Table 1: Cleavage of 75 human light chains.

Enzyme	Recognition*	Nch	Ns	Planned location of site
AfeI	AGCgct	0	0	
AflII	**Cttaag**	**0**	**0**	**HC FR3**
AgeI	Accggt	0	0	
AscI	**GGcgcgcc**	**0**	**0**	**After LC**
BglII	Agatct	0	0	
BsiWI	Cgtacg	0	0	
BspDI	ATcgat	0	0	
BssHII	Gcgcgc	0	0	
BstBI	TTcgaa	0	0	
DraIII	CACNNNgtg	0	0	
EagI	Cggccg	0	0	
FseI	GGCCGGcc	0	0	
FspI	TGCgca	0	0	
HpaI	GTTaac	0	0	
MfeI	**Caattg**	**0**	**0**	**HC FR1**
MluI	Acgcgt	0	0	
NcoI	**Ccatgg**	**0**	**0**	**Heavy chain signal**
NheI	**Gctagc**	**0**	**0**	**HC/anchor linker**
NotI	**GCggccgc**	**0**	**0**	**In linker after HC**
NruI	TCGcga	0	0	
PacI	TTAATtaa	0	0	
PmeI	GTTTaaac	0	0	
PmlI	CACgtg	0	0	
PvuI	CGATcg	0	0	
SacII	CCGCgg	0	0	
SalI	Gtcgac	0	0	
SfiI	**GGCCNNNNnggcc**	**0**	**0**	**Heavy Chain signal**
SgfI	GCGATcgc	0	0	
SnaBI	TACgta	0	0	
StuI	AGGcct	0	0	
XbaI	**Tctaga**	**0**	**0**	**HC FR3**
AatII	GACGTc	1	1	
AclI	AAcgtt	1	1	
AseI	ATtaat	1	1	
BsmI	GAATGCN	1	1	
BspEI	**Tccgga**	**1**	**1**	**HC FR1**
BstXI	**CCANNNNNntgg**	**1**	**1**	**HC FR2**
DrdI	GACNNNNnngtc	1	1	
HindIII	Aagctt	1	1	
PciI	Acatgt	1	1	
SapI	gaagagc	1	1	
ScaI	AGTact	1	1	
SexAI	Accwggt	1	1	
SpeI	Actagt	1	1	
TliI	Ctcgag	1	1	
XhoI	Ctcgag	1	1	
BcgI	cgannnnnntgc	2	2	
BlpI	GCtnagc	2	2	
BssSI	Ctcgtg	2	2	
BstAPI	GCANNNNntgc	2	2	
EspI	GCtnagc	2	2	
KasI	Ggcgcc	2	2	
PflMI	CCANNNNntgg	2	2	
XmnI	GAANNnnttc	2	2	

ApaLI	**Gtgcac**	**3**	**3**	**LC signal seq**
NaeI	GCCggc	3	3	
NgoMI	Gccggc	3	3	
PvuII	CAGctg	3	3	
RsrII	CGgwccg	3	3	
BsrBI	GAGcgg	4	4	
BsrDI	GCAATGNNn	4	4	
BstZ17I	GTAtac	4	4	
EcoRI	Gaattc	4	4	
SphI	GCATGc	4	4	
SspI	AATatt	4	4	
AccI	GTmkac	5	5	
BclI	Tgatca	5	5	
BsmBI	Nnnnnngagacg	5	5	
BsrGI	Tgtaca	5	5	
DraI	TTTaaa	6	6	
NdeI	**CAtatg**	**6**	**6**	**HC FR4**
SwaI	ATTTaaat	6	6	
BamHI	Ggatcc	7	7	
SacI	GAGCTc	7	7	
BciVI	GTATCCNNNNNN	8	8	
BsaBI	GATNNnnatc	8	8	
NsiI	ATGCAt	8	8	
Bsp120I	**Gggccc**	**9**	**9**	**CH1**
ApaI	**GGGCCc**	**9**	**9**	**CH1**
PspOOMI	Gggccc	9	9	
BspHI	Tcatga	9	11	
EcoRV	GATatc	9	9	
AhdI	GACNNNnngtc	11	11	
BbsI	GAAGAC	11	14	
PsiI	TTAtaa	12	12	
BsaI	GGTCTCNnnnn	13	15	
XmaI	Cccggg	13	14	
AvaI	Cycgrg	14	16	
BglI	GCCNNNNnggc	14	17	
AlwNI	CAGNNNctg	16	16	
BspMI	ACCTGC	17	19	
XcmI	CCANNNNNnnnntgg	17	26	
BstEII	**Ggtnacc**	**19**	**22**	**HC FR4**
Sse8387I	CCTGCAgg	20	20	
AvrII	Cctagg	22	22	
HincII	GTYrac	22	22	
BsgI	GTGCAG	27	29	
MscI	TGGcca	30	34	
BseRI	NNnnnnnnnnctcctc	32	35	
Bsu36I	CCtnagg	35	37	
PstI	CTGCAg	35	40	
EciI	nnnnnnnnntccgcc	38	40	
PpuMI	RGgwccy	41	50	
StyI	Ccwwgg	44	73	
Eco0109I	RGgnccy	46	70	
Acc65I	Ggtacc	50	51	
KpnI	GGTACc	50	51	
BpmI	ctccag	53	82	
AvaII	Ggwcc	71	124	

* cleavage occurs in the top strand after the last upper-case base. For REs

that cut palindromic sequences, the lower strand is cut at the symmetrical site.

Table 2: Cleavage of 79 human heavy chains

Enzyme	Recognition	Nch	Ns	Planned location of site
AfeI	AGCgct	0	0	
AflII	**Cttaag**	**0**	**0**	**HC FR3**
AscI	**GGcgcgcc**	**0**	**0**	**After LC**
BsiWI	Cgtacg	0	0	
BspDI	ATcgat	0	0	
BssHII	Gcgcgc	0	0	
FseI	GGCCGGcc	0	0	
HpaI	GTTaac	0	0	
NheI	**Gctagc**	**0**	**0**	**HC Linker**
NotI	**GCggccgc**	**0**	**0**	**In linker, HC/anchor**
NruI	TCGcga	0	0	
NsiI	ATGCAt	0	0	
PacI	TTAATtaa	0	0	
PciI	Acatgt	0	0	
PmeI	GTTTaaac	0	0	
PvuI	CGATcg	0	0	
RsrII	CGgwccg	0	0	
SapI	gaagagc	0	0	
SfiI	**GGCCNNNNnggcc**	**0**	**0**	**HC signal seq**
SgfI	GCGATcgc	0	0	
SwaI	ATTTaaat	0	0	
AclI	AAcgtt	1	1	
AgeI	Accggt	1	1	
AseI	ATtaat	1	1	
AvrII	Cctagg	1	1	
BsmI	GAATGCN	1	1	
BsrBI	GAGcgg	1	1	
BsrDI	GCAATGNNn	1	1	
DraI	TTTaaa	1	1	
FspI	TGCgca	1	1	
HindIII	Aagctt	1	1	
MfeI	**Caattg**	**1**	**1**	**HC FR1**
NaeI	GCCggc	1	1	
NgoMI	Gccggc	1	1	
SpeI	Actagt	1	1	
Acc65I	Ggtacc	2	2	
BstBI	TTcgaa	2	2	
KpnI	GGTACc	2	2	
MluI	Acgcgt	2	2	
NcoI	**Ccatgg**	**2**	**2**	**In HC signal seq**
NdeI	**CAtatg**	**2**	**2**	**HC FR4**
PmlI	CACgtg	2	2	
XcmI	CCANNNNNnnnntgg	2	2	
BcgI	cgannnnnntgc	3	3	
BclI	Tgatca	3	3	
BglI	GCCNNNNnggc	3	3	
BsaBI	GATNNnnatc	3	3	
BsrGI	Tgtaca	3	3	
SnaBI	TACgta	3	3	
Sse8387I	CCTGCAgg	3	3	

ApaLI	**Gtgcac**	**4**	**4**	**LC Signal/FR1**
BspHI	Tcatga	4	4	
BssSI	Ctcgtg	4	4	
PsiI	TTAtaa	4	5	
SphI	GCATGc	4	4	
AhdI	GACNNNnngtc	5	5	
BspEI	**Tccgga**	**5**	**5**	**HC FR1**
MscI	TGGcca	5	5	
SacI	GAGCTc	5	5	
ScaI	AGTact	5	5	
SexAI	Accwggt	5	6	
SspI	AATatt	5	5	
TliI	Ctcgag	5	5	
XhoI	Ctcgag	5	5	
BbsI	GAAGAC	7	8	
BstAPI	GCANNNNntgc	7	8	
BstZ17I	GTAtac	7	7	
EcoRV	GATatc	7	7	
EcoRI	Gaattc	8	8	
BlpI	GCtnagc	9	9	
Bsu36I	CCtnagg	9	9	
DraIII	CACNNNgtg	9	9	
EspI	GCtnagc	9	9	
StuI	AGGcct	9	13	
XbaI	**Tctaga**	**9**	**9**	**HC FR3**
Bsp120I	**Gggccc**	**10**	**11**	**CH1**
ApaI	**GGGCCc**	**10**	**11**	**CH1**
PspOOMI	Gggccc	10	11	
BciVI	GTATCCNNNNNN	11	11	
SalI	Gtcgac	11	12	
DrdI	GACNNNNnngtc	12	12	
KasI	Ggcgcc	12	12	
XmaI	Cccggg	12	14	
BglII	Agatct	14	14	
HincII	GTYrac	16	18	
BamHI	Ggatcc	17	17	
PflMI	CCANNNNntgg	17	18	
BsmBI	Nnnnnngagacg	18	21	
BstXI	**CCANNNNNntgg**	**18**	**19**	**HC FR2**
XmnI	GAANNnnttc	18	18	
SacII	CCGCgg	19	19	
PstI	CTGCAg	20	24	
PvuII	CAGctg	20	22	
AvaI	Cycgrg	21	24	
EagI	Cggccg	21	22	
AatII	GACGTc	22	22	
BspMI	ACCTGC	27	33	
AccI	GTmkac	30	43	
StyI	Ccwwgg	36	49	
AlwNI	CAGNNNctg	38	44	
BsaI	GGTCTCNnnnn	38	44	
PpuMI	RGgwccy	43	46	
BsgI	GTGCAG	44	54	
BseRI	NNnnnnnnnnctcctc	48	60	
EciI	nnnnnnnnntccgcc	52	57	
BstEII	**Ggtnacc**	**54**	**61**	**HC Fr4, 47/79 have one**
Eco0109I	RGgnccy	54	86	

BpmI	ctccag	60	121
AvaII	Ggwcc	71	140

Table 5(~~amended~~): Use of *Fok*I as "Universal Restriction Enzyme"

```
FokI - for dsDNA, | represents sites of cleavage

                          sites of cleavage
     5'-cacGGATGtg--nnnnnnn|nnnnnnn-3'(SEQ ID NO:15)
     3'-gtgCCTACac--nnnnnnnnnnn|nnn-5'(SEQ ID NO:16)
           RECOG
           NITion of FokI

Case I

              5'-...gtg|tatt-actgtgc..Substrate....-3' (SEQ ID NO:17)
                 3'-cac-ataa|tgacacg┐
                                    gtGTAGGcac\
                                5'- caCATCCgtg/(SEQ ID NO:18)

Case II

              5'-...gtgtatt|agac-tgc..Substrate....-3'(SEQ ID NO:19)
                  ┌-cacataa-tctg|acg-5'
        /gtgCCTACac
        \cacGGATGtg-3'(SEQ ID NO:20)

Case III (Case I rotated 180 degrees)

        /gtgCCTACac-5'
        \cacGGATGtg┐
                   gtgtctt|acag-tcc-3' Adapter (SEQ ID NO:21)
              3'-...cacagaa-tgtc|agg..substrate....-5'(SEQ ID NO:22)

Case IV (Case II rotated 180 degrees)
```

```
                                  3'- gtGTAGGcac\  (SEQ ID NO:23)
                                    ┌─caCATCCgtg/
                5'-gag|tctc-actgagc
    Substrate 3'-...ctc-agag|tgactcg...-5'(SEQ ID NO:24)
```

Improved *Fok*I adapters

*Fok*I - for dsDNA, | represents sites of cleavage

Case I
Stem 11, loop 5, stem 11, recognition 17

```
           5'-...catgtg|tatt-actgtgc..Substrate....-3'
              3'-gtacac-ataa|tgacacg─┐          ┌T─┐
                                    gtGTAGGcacG  T
                                 5'- caCATCCgtgc  C
                                              └TT┘
```

Case II
Stem 10, loop 5, stem 10, recognition 18

```
               5'-...gtgtatt|agac-tgctgcc..Substrate....-3'
      ┌T┐        ┌─cacataa-tctg|acgacgg-5'
      T  gtgCCTACac
      C  cacGGATGtg-3'
      └TT┘
```

Case III (Case I rotated 180 degrees)
Stem 11, loop 5, stem 11, recognition 20

```
     ┌ T┐
     T  TgtgCCTACac-5'
     G  AcacGGATGtg─┐
     └TT┘           gtgtctt|acag-tccattctg-3' Adapter
               3'-...cacagaa-tgtc|aggtaagac..substrate....-5'
```

Case IV (Case II rotated 180 degrees)
Stem 11, loop 4, stem 11, recognition 17

```
                                        ┌T┐
                                  3'- gtGTAGGcacc T
                                    ┌─caCATCCgtgg T
               5'-atcgag|tctc-actgagc           └T┘
 Substrate 3'-...tagctc-agag|tgactcg...-5'
```

BseRI

```
                                  | sites of cleavage
     5'-cacGAGGAGnnnnnnnnnn|nnnnn-3'
     3'-gtgctcctcnnnnnnnn|nnnnnnn-5'
           RECOG
           NITion of BseRI
```

Stem 11, loop 5, stem 11, recognition 19

```
          3'-.......gaacat|cg-ttaagccagta.....5'
    ┌T-T┐           cttgta-gc|aattcggtcat-3'
    C   GCTGAGGAGTC-┘
    T   cgactcctcag-5'   An adapter for BseRI to cleave the substrate above.
    └T──┘
```

Table 8: Matches to URE FR3 adapters in 79 human HC.

A. List of Heavy-chains genes sampled

AF008566	af103343	HSA235676	HSU92452	HSZ93860
AF035043	AF103367	HSA235675	HSU94412	HSZ93863
AF103026	AF103368	HSA235674	HSU94415	MCOMFRAA
af103033	AF103369	HSA235673	HSU94416	MCOMFRVA
AF103061	AF103370	HSA240559	HSU94417	S82745
Af103072	af103371	HSCB201	HSU94418	S82764
af103078	AF103372	HSIGGVHC	HSU96389	S83240
AF103099	AF158381	HSU44791	HSU96391	SABVH369
AF103102	E05213	HSU44793	HSU96392	SADEIGVH
AF103103	E05886	HSU82771	HSU96395	SAH2IGVH
AF103174	E05887	HSU82949	HSZ93849	SDA3IGVH
AF103186	HSA235661	HSU82950	HSZ93850	SIGVHTTD
af103187	HSA235664	HSU82952	HSZ93851	SUK4IGVH
AF103195	HSA235660	HSU82961	HSZ93853	
af103277	HSA235659	HSU86522	HSZ93855	
af103286	HSA235678	HSU86523	HSZ93857	
AF103309	HSA235677			

Table 8 B. Testing all distinct GLGs from bases 89.1 to 93.2 of the heavy variable domain

Id	Nb	0	1	2	3	4		SEQ ID NO:
1	38	15	11	10	0	2	Seq1 gtgtattactgtgc	25
2	19	7	6	4	2	0	Seq2 gtAtattactgtgc	26
3	1	0	0	1	0	0	Seq3 gtgtattactgtAA	27
4	7	1	5	1	0	0	Seq4 gtgtattactgtAc	28
5	0	0	0	0	0	0	Seq5 Ttgtattactgtgc	29
6	0	0	0	0	0	0	Seq6 TtgtatCactgtgc	30
7	3	1	0	1	1	0	Seq7 ACAtattactgtgc	31
8	2	0	2	0	0	0	Seq8 ACgtattactgtgc	32
9	9	2	2	4	1	0	Seq9 ATgtattactgtgc	33
Group		26	26	21	4	2		
Cumulative		26	52	73	77	79		

Table 8C Most important URE recognition seqs in FR3 Heavy

1	VHSzy1	GTGtattactgtgc	(ON_SHC103)	(SEQ ID NO:25)
2	VHSzy2	GTAtattactgtgc	(ON_SHC323)	(SEQ ID NO:26)
3	VHSzy4	GTGtattactgtac	(ON_SHC349)	(SEQ ID NO:28)
4	VHSzy9	ATGtattactgtgc	(ON_SHC5a)	(SEQ ID NO:33)

Table 8D, testing 79 human HC V genes with four probes

Number of sequences..... 79
Number of bases..... 29143

		Number of mismatches						
Id	Best	0	1	2	3	4	5	
1	39	15	11	10	1	2	0	Seq1 gtgtattactgtgc (SEQ ID NO:25)
2	22	7	6	5	3	0	1	Seq2 gtAtattactgtgc (SEQ ID NO:26)
3	7	1	5	1	0	0	0	Seq4 gtgtattactgtAc (SEQ ID NO:28)
4	11	2	4	4	1	0	0	Seq9 ATgtattactgtgc (SEQ ID NO:33)
Group		25	26	20	5	2		
Cumulative		25	51	71	76	78		

One sequence has five mismatches with sequences 2, 4, and 9; it is scored as best for 2.

Id is the number of the adapter.

Best is the number of sequence for which the identified adapter was the best available.

The rest of the table shows how well the sequences match the adapters. For example, there are 11 sequences that match VHSzy1(Id=1) with 2 mismatches and are worse for all other adapters. In this sample, 90% come within 2 bases of one of the four adapters.

Table 130: PCR primers for amplification of human Ab genes

(HuIgMFOR) 5'-tgg aag agg cac gtt ctt ttc ttt-3'

!(HuIgMFOREtop) 5'-aaa gaa aag aac gtg cct ctt cca-3' = reverse complement

(HuCkFOR) 5'-aca ctc tcc cct gtt gaa gct ctt-3'

(HuCL2FOR) 5'-tga aca ttc tgt agg ggc cac tg-3'

(HuCL7FOR) 5'-aga gca ttc tgc agg ggc cac tg-3'

! Kappa

(CKForeAsc) 5'-acc gcc tcc acc ggg cgc gcc tta tta aca ctc tcc cct gtt-gaa gct ctt-3'

(CL2ForeAsc) 5'-acc gcc tcc acc ggg cgc gcc tta tta tga aca ttc tgt-agg ggc cac tg-3'

(CL7ForeAsc) 5'-acc gcc tcc acc ggg cgc gcc tta tta aga gca ttc tgc-agg ggc cac tg-3'

Table 195: Human GLG FR3 sequences

! VH1

! 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80

agg gtc acc atg acc agg gac acg tcc atc agc aca gcc tac atg
! 81 82 82a 82b 82c 83 84 85 86 87 88 89 90 91 92
gag ctg agc agg ctg aga tct gac gac acg gcc gtg tat tac tgt
! 93 94 95
gcg aga ga ! 1-02# 1
aga gtc acc att acc agg gac aca tcc gcg agc aca gcc tac atg
gag ctg agc agc ctg aga tct gaa gac acg gct gtg tat tac tgt
gcg aga ga ! 1-03# 2
aga gtc acc atg acc agg aac acc tcc ata agc aca gcc tac atg
gag ctg agc agc ctg aga tct gag gac acg gcc gtg tat tac tgt
gcg aga gg ! 1-08# 3
aga gtc acc atg acc aca gac aca tcc acg agc aca gcc tac atg
gag ctg agg agc ctg aga tct gac gac acg gcc gtg tat tac tgt
gcg aga ga ! 1-18# 4
aga gtc acc atg acc gag gac aca tct aca gac aca gcc tac atg
gag ctg agc agc ctg aga tct gag gac acg gcc gtg tat tac tgt
gca aca ga ! 1-24# 5
aga gtc acc att acc agg gac agg tct atg agc aca gcc tac atg
gag ctg agc agc ctg aga tct gag gac aca gcc atg tat tac tgt
gca aga ta ! 1-45# 6
aga gtc acc atg acc agg gac acg tcc acg agc aca gtc tac atg
gag ctg agc agc ctg aga tct gag gac acg gcc gtg tat tac tgt
gcg aga ga ! 1-46# 7

aga gtc acc att acc agg gac atg tcc aca agc aca gcc tac atg
gag ctg agc agc ctg aga tcc gag gac acg gcc gtg tat tac tgt
gcg gca ga ! 1-58# 8
aga gtc acg att acc gcg gac gaa tcc acg agc aca gcc tac atg
gag ctg agc agc ctg aga tct gag gac acg gcc gtg tat tac tgt
gcg aga ga ! 1-69# 9
aga gtc acg att acc gcg gac aaa tcc acg agc aca gcc tac atg
gag ctg agc agc ctg aga tct gag gac acg gcc gtg tat tac tgt
gcg aga ga ! 1-e# 10
aga gtc acc ata acc gcg gac acg tct aca gac aca gcc tac atg
gag ctg agc agc ctg aga tct gag gac acg gcc gtg tat tac tgt
gca aca ga ! 1-f# 11
! VH2
agg ctc acc atc acc aag gac acc tcc aaa aac cag gtg gtc ctt
aca atg acc aac atg gac cct gtg gac aca gcc aca tat tac tgt
gca cac aga c! 2-05# 12
agg ctc acc atc tcc aag gac acc tcc aaa agc cag gtg gtc ctt
acc atg acc aac atg gac cct gtg gac aca gcc aca tat tac tgt
gca cgg ata c! 2-26# 13
agg ctc acc atc tcc aag gac acc tcc aaa aac cag gtg gtc ctt
aca atg acc aac atg gac cct gtg gac aca gcc acg tat tac tgt
gca cgg ata c! 2-70# 14
! VH3
cga ttc acc atc tcc aga gac aac gcc aag aac tca ctg tat ctg
caa atg aac agc ctg aga gcc gag gac acg gct gtg tat tac tgt
gcg aga ga ! 3-07# 15
cga ttc acc atc tcc aga gac aac gcc aag aac tcc ctg tat ctg
caa atg aac agt ctg aga gct gag gac acg gcc ttg tat tac tgt
gca aaa gat a! 3-09#16
cga ttc acc atc tcc agg gac aac gcc aag aac tca ctg tat ctg
caa atg aac agc ctg aga gcc gag gac acg gcc gtg tat tac tgt
gcg aga ga ! 3-11# 17
cga ttc acc atc tcc aga gaa aat gcc aag aac tcc ttg tat ctt
caa atg aac agc ctg aga gcc ggg gac acg gct gtg tat tac tgt
gca aga ga ! 3-13# 18
aga ttc acc atc tca aga gat gat tca aaa aac acg ctg tat ctg
caa atg aac agc ctg aaa acc gag gac aca gcc gtg tat tac tgt
acc aca ga ! 3-15# 19
cga ttc acc atc tcc aga gac aac gcc aag aac tcc ctg tat ctg

caa atg aac agt ctg aga gcc gag gac acg gcc ttg tat cac tgt
gcg aga ga ! 3-20# 20
cga ttc acc atc tcc aga gac aac gcc aag aac tca ctg tat ctg
caa atg aac agc ctg aga gcc gag gac acg gct gtg tat tac tgt
gcg aga ga ! 3-21# 21
cgg ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctg
caa atg aac agc ctg aga gcc gag gac acg gcc gta tat tac tgt
gcg aaa ga ! 3-23# 22
cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctg
caa atg aac agc ctg aga gct gag gac acg gct gtg tat tac tgt
gcg aaa ga ! 3-30# 23
cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctg
caa atg aac agc ctg aga gct gag gac acg gct gtg tat tac tgt
gcg aga ga ! 3303# 24
cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctg
caa atg aac agc ctg aga gct gag gac acg gct gtg tat tac tgt
gcg aaa ga ! 3305# 25
cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctg
caa atg aac agc ctg aga gcc gag gac acg gct gtg tat tac tgt
gcg aga ga ! 3-33# 26
cga ttc acc atc tcc aga gac aac agc aaa aac tcc ctg tat ctg
caa atg aac agt ctg aga act gag gac acc gcc ttg tat tac tgt
gca aaa gat a! 3-43#27
cga ttc acc atc tcc aga gac aat gcc aag aac tca ctg tat ctg
caa atg aac agc ctg aga gac gag gac acg gct gtg tat tac tgt
gcg aga ga ! 3-48# 28
aga ttc acc atc tca aga gat ggt tcc aaa agc atc gcc tat ctg
caa atg aac agc ctg aaa acc gag gac aca gcc gtg tat tac tgt
act aga ga ! 3-49# 29
cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctt
caa atg aac agc ctg aga gcc gag gac acg gcc gtg tat tac tgt
gcg aga ga ! 3-53# 30
aga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctt
caa atg ggc agc ctg aga gct gag gac atg gct gtg tat tac tgt
gcg aga ga ! 3-64# 31
aga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctt
caa atg aac agc ctg aga gct gag gac acg gct gtg tat tac tgt
gcg aga ga ! 3-66# 32
aga ttc acc atc tca aga gat gat tca aag aac tca ctg tat ctg

caa atg aac agc ctg aaa acc gag gac acg gcc gtg tat tac tgt
gct aga ga ! 3-72# 33
agg ttc acc atc tcc aga gat gat tca aag aac acg gcg tat ctg
caa atg aac agc ctg aaa acc gag gac acg gcc gtg tat tac tgt
act aga ca ! 3-73# 34
cga ttc acc atc tcc aga gac aac gcc aag aac acg ctg tat ctg
caa atg aac agt ctg aga gcc gag gac acg gct gtg tat tac tgt
gca aga ga ! 3-74# 35
aga ttc acc atc tcc aga gac aat tcc aag aac acg ctg cat ctt
caa atg aac agc ctg aga gct gag gac acg gct gtg tat tac tgt
aag aaa ga ! 3-d# 36
! VH4
cga gtc acc ata tca gta gac aag tcc aag aac cag ttc tcc ctg
aag ctg agc tct gtg acc gcc gcg gac acg gcc gtg tat tac tgt
gcg aga ga ! 4-04# 37
cga gtc acc atg tca gta gac acg tcc aag aac cag ttc tcc ctg
aag ctg agc tct gtg acc gcc gtg gac acg gcc gtg tat tac tgt
gcg aga aa ! 4-28# 38
cga gtt acc ata tca gta gac acg tct aag aac cag ttc tcc ctg
aag ctg agc tct gtg act gcc gcg gac acg gcc gtg tat tac tgt
gcg aga ga ! 4301# 39
cga gtc acc ata tca gta gac agg tcc aag aac cag ttc tcc ctg
aag ctg agc tct gtg acc gcc gcg gac acg gcc gtg tat tac tgt
gcc aga ga ! 4302# 40
cga gtt acc ata tca gta gac acg tcc aag aac cag ttc tcc ctg
aag ctg agc tct gtg act gcc gca gac acg gcc gtg tat tac tgt
gcc aga ga ! 4304# 41
cga gtt acc ata tca gta gac acg tct aag aac cag ttc tcc ctg
aag ctg agc tct gtg act gcc gcg gac acg gcc gtg tat tac tgt
gcg aga ga ! 4-31# 42
cga gtc acc ata tca gta gac acg tcc aag aac cag ttc tcc ctg
aag ctg agc tct gtg acc gcc gcg gac acg gct gtg tat tac tgt
gcg aga ga ! 4-34# 43
cga gtc acc ata tcc gta gac acg tcc aag aac cag ttc tcc ctg
aag ctg agc tct gtg acc gcc gca gac acg gct gtg tat tac tgt
gcg aga ca ! 4-39# 44
cga gtc acc ata tca gta gac acg tcc aag aac cag ttc tcc ctg
aag ctg agc tct gtg acc gct gcg gac acg gcc gtg tat tac tgt
gcg aga ga ! 4-59# 45

cga gtc acc ata tca gta gac acg tcc aag aac cag ttc tcc ctg
aag ctg agc tct gtg acc gct gcg gac acg gcc gtg tat tac tgt
gcg aga ga ! 4-61# 46
cga gtc acc ata tca gta gac acg tcc aag aac cag ttc tcc ctg
aag ctg agc tct gtg acc gcc gca gac acg gcc gtg tat tac tgt
gcg aga ga ! 4-b# 47
! VH5
cag gtc acc atc tca gcc gac aag tcc atc agc acc gcc tac ctg
cag tgg agc agc ctg aag gcc tcg gac acc gcc atg tat tac tgt
gcg aga ca ! 5-51# 48
cac gtc acc atc tca gct gac aag tcc atc agc act gcc tac ctg
cag tgg agc agc ctg aag gcc tcg gac acc gcc atg tat tac tgt
gcg aga ! 5-a# 49
! VH6
cga ata acc atc aac cca gac aca tcc aag aac cag ttc tcc ctg
cag ctg aac tct gtg act ccc gag gac acg gct gtg tat tac tgt
gca aga ga ! 6-1# 50
! VH7
cgg ttt gtc ttc tcc ttg gac acc tct gtc agc acg gca tat ctg
cag atc tgc agc cta aag gct gag gac act gcc gtg tat tac tgt
gcg aga ga ! 74.1# 51

Table 250: REdaptors, Extenders, and Bridges used for Cleavage and Capture of Human Heavy Chains in FR3.

A: HpyCH4V Probes of actual human HC genes

!HpyCH4V in FR3 of human HC, bases 35-56; only those with TGca site

TGca;10,

RE recognition:tgca of length 4 is expected at 10

1 6-1 **agttctccctgcagctgaactc**

2	3-11,3-07,3-21,3-72,3-48	**cactgtatctgcaaatgaacag**
3	3-09,3-43,3-20	ccctgtatctgcaaatgaacag
4	5-51	**ccgcctacctgcagtggagcag**
5	3-15,3-30,3-30.5,3-30.3,3-74,3-23,3-33	**cgctgtatctgcaaatgaacag**
6	7-4.1	cggcatatctgcagatctgcag
7	3-73	cggcgtatctgcaaatgaacag
8	5-a	ctgcctacctgcagtggagcag
9	3-49	tcgcctatctgcaaatgaacag

B: HpyCH4V REdaptors, Extenders, and Bridges

B.1 REdaptors

```
! Cutting HC lower strand:
! TmKeller for 100 mM NaCl, zero formamide
! Edapters for cleavage                                    Tm^W      Tm^K
(ON_HCFR36-1)       5'-agttctcccTGCAgctgaactc-3'          68.0      64.5
(ON_HCFR36-1A)        5'-ttctcccTGCAgctgaactc-3'          62.0      62.5
(ON_HCFR36-1B)        5'-ttctcccTGCAgctgaac-3'            56.0      59.9
(ON_HCFR33-15)      5'-cgctgtatcTGCAaatgaacag-3'          64.0      60.8
(ON_HCFR33-15A)       5'-ctgtatcTGCAaatgaacag-3'          56.0      56.3
(ON_HCFR33-15B)       5'-ctgtatcTGCAaatgaac-3'            50.0      53.1
(ON_HCFR33-11)      5'-cactgtatcTGCAaatgaacag-3'          62.0      58.9
(ON_HCFR35-51)      5'-ccgcctaccTGCAgtggagcag-3'          74.0      70.1
!
```

B.2 Segment of synthetic 3-23 gene into which captured CDR3 is to be cloned

```
!                     XbaI...
!D323*  cgCttcacTaag tcT aga gac aaC tcT aag aaT acT ctC taC
!       scab........ designed gene 3-23 gene.................
!
!    HpyCH4V
!     .. ..            AflII...
!    Ttg caG atg aac agc TtA agG . . .
!    ............................ . . .
!
```

B.3 Extender and Bridges

```
! Extender (bottom strand):
!
(ON_HCHpyEx01)  5'-cAAgTAgAgAgTATTcTTAgAgTTgTcTcTAgAcTTAgTgAAgcg-3'
! ON_HCHpyEx01 is the reverse complement of
! 5'-cgCttcacTaag tcT aga gac aaC tcT aag aaT acT ctC taC Ttg -3'
!
! Bridges (top strand, 9-base overlap):
```

!

(ON_HCHpyBr016-1) 5'-cgCttcacTaag tcT aga gac aaC tcT aag-
aaT acT ctC taC **Ttg** CAgctgaac-3' {3'-term C is blocked}

!

! 3-15 et al. + 3-11

(ON_HCHpyBr023-15) 5'-cgCttcacTaag tcT aga gac aaC tcT aag-
aaT acT ctC taC **Ttg** CAaatgaac-3' {3'-term C is blocked}

!

! 5-51

(ON_HCHpyBr045-51) 5'-cgCttcacTaag tcT aga gac aaC tcT aag-
aaT acT ctC taC **Ttg** CAgtggagc-3' {3'-term C is blocked}

!

! PCR primer (top strand)

!

(ON_HCHpyPCR) 5'-cgCttcacTaag tcT aga gac-3'

!

C: *BlpI* Probes from human HC GLGs

1	1-58,1-03,1-08,1-69,1-24,1-45,1-46,1-f,1-e	acatggaGCTGAGCagcctgag
2	1-02	acatggaGCTGAGCaggctgag
3	1-18	acatggagctgaggagcctgag
4	5-51,5-a	acctgcagtggagcagcctgaa
5	3-15,3-73,3-49,3-72	atctgcaaatgaacagcctgaa
6	3303,3-33,3-07,3-11,3-30,3-21,3-23,3305,3-48	atctgcaaatgaacagcctgag
7	3-20,3-74,3-09,3-43	atctgcaaatgaacagtctgag
8	74.1	atctgcagatctgcagcctaaa
9	3-66,3-13,3-53,3-d	atcttcaaatgaacagcctgag
10	3-64	atcttcaaatgggcagcctgag
11	4301,4-28,4302,4-04,4304,4-31,4-34,4-39,4-59,4-61,4-b	ccctgaaGCTGAGCtctgtgac
12	6-1	ccctgcagctgaactctgtgac
13	2-70,2-05	tccttacaatgaccaacatgga
14	2-26	tccttaccatgaccaacatgga

D: *BlpI* REdaptors, Extenders, and Bridges

D.1 REdaptors

		T_m^W	T_mK
(BlpF3HC1-58)	5'-ac atg gaG **CTG AGC** agc ctg ag-3'	70	66.4
(BlpF3HC6-1)	5'-cc ctg aag ctg agc tct gtg ac-3'	70	66.4

! BlpF3HC6-1 matches 4-30.1, not 6-1.

D.2 Segment of synthetic 3-23 gene into which captured CDR3 is to be cloned

```
!                                                                    BlpI
!                    XbaI...                                         . ... ...
!D323*  cgCttcacTaag TCT AGA gac aaC tcT aag aaT acT ctC taC Ttg caG atg aac
!
!                    AflII...
!                   agC TTA AGG
```

D.3 Extender and Bridges

```
! Bridges
(BlpF3Br1) 5'-cgCttcacTcag tcT aga gaT aaC AGT aaA aaT acT TtG-
                taC Ttg caG Ctg a|GC agc ctg-3'
(BlpF3Br2) 5'-cgCttcacTcag tcT aga gaT aaC AGT aaA aaT acT TtG-
                taC Ttg caG Ctg a|gc tct gtg-3'
!                                  | lower strand is cut here
! Extender
(BlpF3Ext) 5'-
TcAgcTgcAAgTAcAAAgTATTTTTAcTgTTATcTcTAgAcTgAgTgAAgcg-3'
! BlpF3Ext is the reverse complement of:
! 5'-cgCttcacTcag tcT aga gaT aaC AGT aaA aaT acT TtG taC Ttg caG Ctg a-3'
!
(BlpF3PCR) 5'-cgCttcacTcag tcT aga gaT aaC-3'
```

E: *Hpy*CH4III Distinct GLG sequences surrounding site, bases 77-98

1	102#1,118#4,146#7,169#9,1e#10,311#17,353#30,404#37,4301	ccgtgtattactgtgcgagaga
2	103#2,307#15,321#21,3303#24,333#26,348#28,364#31,366#32	ctgtgtattactgtgcgagaga
3	108#3	ccgtgtattactgtgcgagagg
4	124#5,1f#11	ccgtgtattactgtgcaacaga
5	145#6	ccatgtattactgtgcaagata
6	158#8	ccgtgtattactgtgcggcaga
7	205#12	ccacatattactgtgcacacag
8	226#13	ccacatattactgtgcacggat
9	270#14	ccacgtattactgtgcacggat
10	309#16,343#27	ccttgtattactgtgcaaaaga
11	313#18,374#35,61#50	ctgtgtattactgtgcaagaga
12	315#19	ccgtgtattactgtaccacaga
13	320#20	ccttgtatcactgtgcgagaga
14	323#22	ccgtatattactgtgcgaaaga
15	330#23,3305#25	ctgtgtattactgtgcgaaaga
16	349#29	ccgtgtattactgtactagaga
17	372#33	ccgtgtattactgtgctagaga
18	373#34	ccgtgtattactgtactagaca
19	3d#36	ctgtgtattactgtaagaaaga
20	428#38	ccgtgtattactgtgcgagaaa
21	4302#40,4304#41	ccgtgtattactgtgccagaga
22	439#44	ctgtgtattactgtgcgagaca
23	551#48	ccatgtattactgtgcgagaca

F: *HpyCH4III* REdaptors, Extenders, and Bridges

F.1 REdaptors

```
! ONs for cleavage of HC(lower) in FR3(bases 77-97)
! For cleavage with HpyCH4III, Bst4CI, or TaaI
! cleavage is in lower chain before base 88.
!                      77 788 888 888 889 999 999 9
!                      78 901 234 567 890 123 456 7          Tm^W        Tm^K
(H43.77.97.1-02#1)  5'-cc gtg tat tAC TGT gcg aga g-3'      64          62.6
(H43.77.97.1-03#2)  5'-ct gtg tat tAC TGT gcg aga g-3'      62          60.6
(H43.77.97.108#3)   5'-cc gtg tat tAC TGT gcg aga g-3'      64          62.6
(H43.77.97.323#22)  5'-cc gta tat tac tgt gcg aaa g-3'      60          58.7
(H43.77.97.330#23)  5'-ct gtg tat tac tgt gcg aaa g-3'      60          58.7
(H43.77.97.439#44)  5'-ct gtg tat tac tgt gcg aga c-3'      62          60.6
(H43.77.97.551#48)  5'-cc atg tat tac tgt gcg aga c-3'      62          60.6
(H43.77.97.5a#49)   5'-cc atg tat tAC TGT gcg aga -3'       58          58.3
```

F.2 Extender and Bridges

```
! XbaI and AflII sites in bridges are bunged
(H43.XABr1) 5'-ggtgtagtga-
  |TCT|AGt|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-
  |aac|agC|TTt|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat tgt gcg aga-3'
(H43.XABr2) 5'-ggtgtagtga-
  |TCT|AGt|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-
  |aac|agC|TTt|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat tgt gcg aaa-3'
(H43.XAExt) 5'-ATAgTAgAcT gcAgTgTccT cAgcccTTAA gcTgTTcATc TgcAAgTAgA-
               gAgTATTcTT AgAgTTgTcT cTAgATcAcT AcAcc-3'
!H43.XAExt is the reverse complement of
! 5'-ggtgtagtga-
!  |TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-
!  |aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat -3'

(H43.XAPCR)  5'-ggtgtagtga |TCT|AGA|gac|aac-3'
! XbaI and AflII sites in bridges are bunged
(H43.ABr1) 5'-ggtgtagtga-
  |aac|agC|TTt|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat tgt gcg aga-3'
(H43.ABr2) 5'-ggtgtagtga-
  |aac|agC|TTt|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat tgt gcg aaa-3'
(H43.AExt) 5'-ATAgTAgAcTgcAgTgTccTcAgcccTTAAgcTgTTTcAcTAcAcc-3'
```

!(H43.AExt) is the reverse complement of 5'-ggtgtagtga-

! |aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat -3'

(H43.APCR) 5'-ggtgtagtga |aac|agC|TTA|AGg|gct|g-3'

Table 510

```
(FOKIact)     5'-cAcATccgTg TTgTT cAcggATgTg-3'

(VHEx881) 5'-AATAgTAgAc TgcAgTgTcc TcAgcccTTA AgcTgTTcAT cTgcAAgTAg-
             AgAgTATTcT TAgAgTTgTc TcTAgAcTTA gTgAAgcg-3'
! note that VHEx881 is the reverse complement of the ON below
!          [RC] 5'-cgCttcacTaag-
!                  Scab........
!                  Synthetic 3-23 as in Table 206
!                  |TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-
!                   XbaI...
!                  |aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|t-3'
!                        AflII...
(VHBA881)       5'-cgCttcacTaag-
                  |TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-
                  |aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|tgt gcg ag-3'
(VHBB881)       5'-cgCttcacTaag-
```

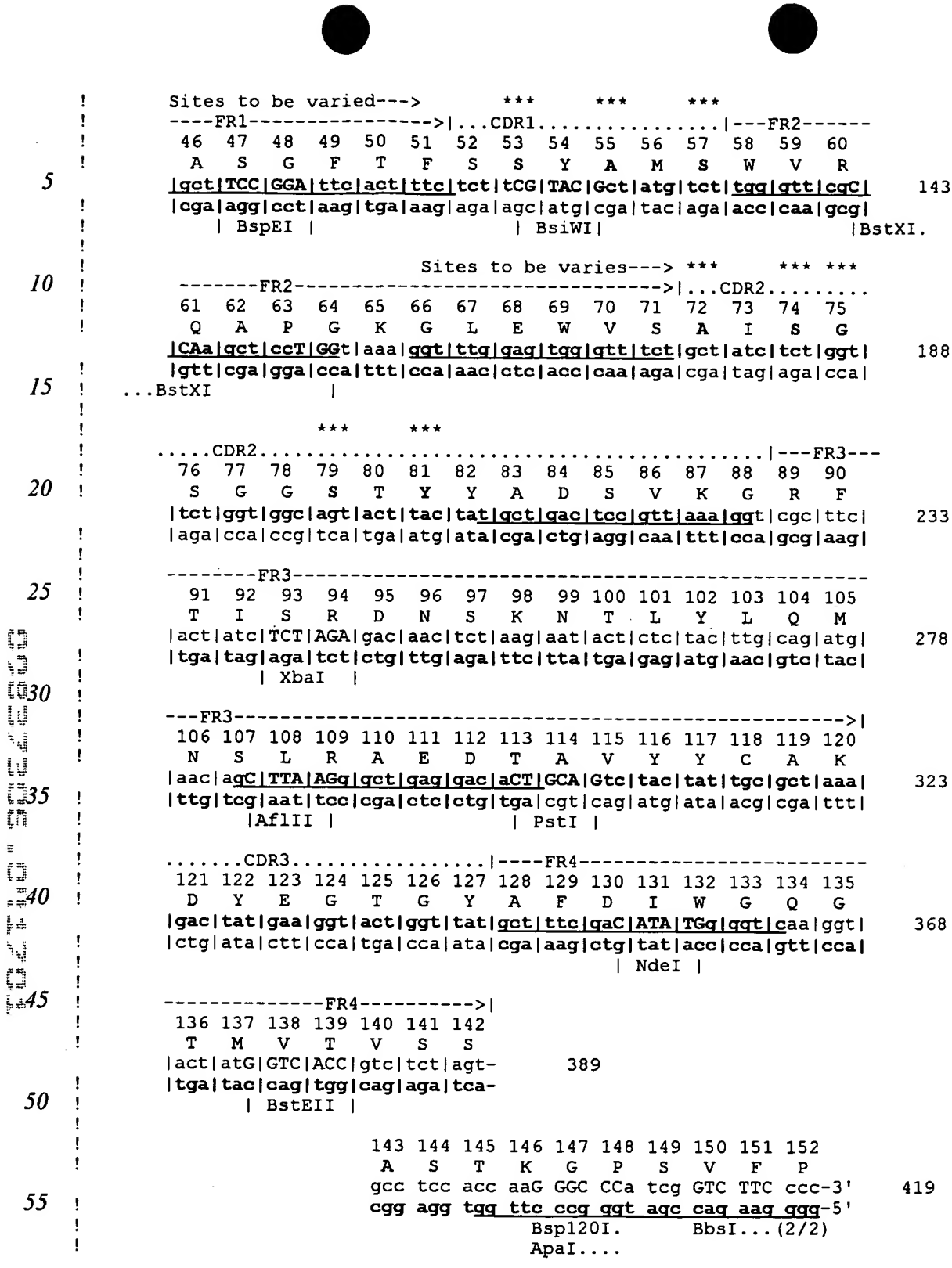
|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-
|aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|tgt Acg ag-3'

(VH881PCR) 5'-cgCttcacTaag|TCT|AGA|gac|aac -3'

Table 600: V3-23 VH framework with variegated codons shown

```
                                  17  18  19  20  21  22
                                   A   Q   P   A   M   A
                5'-ctg tct qaa cG GCC cag ccG GCC atg gcc     29
                3'-gac aga ctt gc cgg gtc ggc cgg tac cgg
                   Scab.........SfiI.............
                                       NgoMI...
                                             NcoI....

                                  FR1(DP47/V3-23)---------------
                                  23  24  25  26  27  28  29  30
                                   E   V   Q   L   L   E   S   G
                                  gaa|gtt|CAA|TTG|tta|gag|tct|ggt|     53
                                  ctt|caa|gtt|aac|aat|ctc|aga|cca|
                                        | MfeI  |
          --------------FR1-----------------------------------------
           31  32  33  34  35  36  37  38  39  40  41  42  43  44  45
            G   G   L   V   Q   P   G   G   S   L   R   L   S   C   A
          |ggc|ggt|ctt|gtt|cag|cct|ggt|ggt|tct|tta|cgt|ctt|tct|tgc|gct|     98
          |ccg|cca|gaa|caa|gtc|gga|cca|cca|aga|aat|gca|gaa|aga|acg|cga|
```


Sites to be varied---> *** *** ***
----FR1----------->|...CDR1.....|---FR2------
46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
A S G F T F S S Y A M S W V R
|gct|TCC|GGA|ttc|act|ttc|tct|tCG|TAC|Gct|atg|tct|tgg|gtt|cgC| 143
|cga|agg|cct|aag|tga|aag|aga|agc|atg|cga|tac|aga|acc|caa|gcg|
| BspEI | | BsiWI| |BstXI.
Sites to be varies---> *** *** ***
-------FR2---------->|...CDR2.....
61 62 63 64 65 66 67 68 69 70 71 72 73 74 75
Q A P G K G L E W V S A I S G
|CAa|gct|ccT|GGt|aaa|ggt|ttg|gag|tgg|gtt|tct|gct|atc|tct|ggt| 188
|gtt|cga|gga|cca|ttt|cca|aac|ctc|acc|caa|aga|cga|tag|aga|cca|
...BstXI |
*** ***
.....CDR2.....|---FR3---
76 77 78 79 80 81 82 83 84 85 86 87 88 89 90
S G G S T Y Y A D S V K G R F
|tct|ggt|ggc|agt|act|tac|tat|gct|gac|tcc|gtt|aaa|ggt|cgc|ttc| 233
|aga|cca|ccg|tca|tga|atg|ata|cga|ctg|agg|caa|ttt|cca|gcg|aag|
--------FR3----------
91 92 93 94 95 96 97 98 99 100 101 102 103 104 105
T I S R D N S K N T L Y L Q M
|act|atc|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg| 278
|tga|tag|aga|tct|ctg|ttg|aga|ttc|tta|tga|gag|atg|aac|gtc|tac|
| XbaI |
---FR3---------->|
106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
N S L R A E D T A V Y Y C A K
|aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|tgc|gct|aaa| 323
|ttg|tcg|aat|tcc|cga|ctc|ctg|tga|cgt|cag|atg|ata|acg|cga|ttt|
|AflII | | PstI |
.....CDR3.....|----FR4----------
121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
D Y E G T G Y A F D I W G Q G
|gac|tat|gaa|ggt|act|ggt|tat|gct|ttc|gaC|ATA|TGg|ggt|caa|ggt| 368
|ctg|ata|ctt|cca|tga|cca|ata|cga|aag|ctg|tat|acc|cca|gtt|cca|
| NdeI |
----------FR4---------->|
136 137 138 139 140 141 142
T M V T V S S
|act|atG|GTC|ACC|gtc|tct|agt- 389
|tga|tac|cag|tgg|cag|aga|tca-
| BstEII |
143 144 145 146 147 148 149 150 151 152
A S T K G P S V F P
gcc tcc acc aaG GGC CCa tcg GTC TTC ccc-3' 419
cgg agg tgg ttc ccg ggt agc cag aag ggg-5'
Bsp120I. BbsI...(2/2)
ApaI....
(SFPRMET) 5'-ctg tct gaa cG GCC cag ccG-3'
(TOPFR1A) 5'-ctg tct gaa cG GCC cag ccG GCC atg gcc-
gaa|gtt|CAA|TTG|tta|gag|tct|ggt|-
|ggc|ggt|ctt|gtt|cag|cct|ggt|ggt|tct|tta-3'
(BOTFR1B) 3'-caa|gtc|gga|cca|cca|aga|aat|gca|gaa|aga|acg|cga|-
|cga|agg|cct|aag|tga|aag-5' ! bottom strand

```
(BOTFR2)    3'-acc|caa|gcg|-
              |gtt|cga|gga|cca|ttt|cca|aac|ctc|acc|caa|aga|-5' ! bottom strand
(BOTFR3)    3'-  a|cga|ctg|agg|caa|ttt|cca|gcg|aag|-
              |tga|tag|aga|tct|ctg|ttg|aga|ttc|tta|tga|gag|atg|aac|gtc|tac|-
          |ttg|tcg|aat|tcc|cga|ctc|ctg|tga-5'
(F06)         5'-gC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|tgc|gct|aaa|-
        |gac|tat|gaa|ggt|act|ggt|tat|gct|ttc|gaC|ATA|TGg|ggt|c-3'
(BOTFR4)    3'-cga|aag|ctg|tat|acc|cca|gtt|cca|-
              |tga|tac|cag|tgg|cag|aga|tca-
                  cgg agg tgg ttc ccg ggt agc cag aag ggg-5' ! bottom strand
(BOTPRCPRIM)            3'-gg ttc ccg ggt agc cag aag ggg-5'
!
! CDR1 diversity
!
(ON-vgC1)   5'-|gct|TCC|GGA|ttc|act|ttc|tct|<1>|TAC|<1>|atg|<1>|-
!                                        CDR1.................6859
              |tgg|gtt|cgC|CAa|gct|ccT|GG-3'
!
!<1> stands for an equimolar mix of {ADEFGHIKLMNPQRSTVWY}; no C
!                                  (this is not a sequence)
!
! CDR2 diversity
!
(ON-vgC2)   5'-ggt|ttg|gag|tgg|gtt|tct|<2>|atc|<2>|<3>|-
!                                     CDR2...........
                |tct|ggt|ggc|<1>|act|<1>|tat|gct|gac|tcc|gtt|aaa|gg-3'
!               CDR2.................................................
! <1> is an equimolar mixture of {ADEFGHIKLMNPQRSTVWY}; no C
! <2> is an equimolar mixture of {YRWVGS}; no ACDEFHIKLMNPQT
! <3> is an equimolar mixture of {PS}; no ACDEFGHIKLMNQRTVWY
```

Table 800 **(new)**

The following list of enzymes was taken from
http://rebase.neb.com/cgi-bin/asymmlist.

I have removed the enzymes that a) cut within the recognition, b) cut on both sides of the recognition, or c) have fewer than 2 bases between recognition and closest cut site.

REBASE Enzymes
04/13/2001

Type II restriction enzymes with asymmetric recognition sequences:

Enzymes	Recognition Sequence	Isoschizomers	Suppliers		
AarI	CACCTGCNNNN^NNNN_	-	y		
AceIII	CAGCTCNNNNNNN^NNNN_	-	-		
Bbr7I	GAAGACNNNNNNN^NNNN_	-	-		
BbvI	GCAGCNNNNNNNN^NNNN_		y		
BbvII	GAAGACNN^NNNN_				
Bce83I	CTTGAGNNNNNNNNNNNNNN_NN^	-	-		
BceAI	ACGGCNNNNNNNNNNNN^NN_	-	y		
BcefI	ACGGCNNNNNNNNNNNN^N_	-	-		
BciVI	GTATCCNNNNN_N^	BfuI	y		
BfiI	ACTGGGNNNN_N^	BmrI	y		
BinI	GGATCNNNN^N_				
BscAI	GCATCNNNN^NN_	-	-		
BseRI	GAGGAGNNNNNNNN_NN^	-	y		
BsmFI	GGGACNNNNNNNNNN^NNNN_	BspLU11III	y		
BspMI	ACCTGCNNNN^NNNN_	Acc36I	y		
EciI	GGCGGANNNNNNNNN_NN^	-	y		
Eco57I	CTGAAGNNNNNNNNNNNNNNN_NN^	BspKT5I	y		
FauI	CCCGCNNNN^NN_	BstFZ438I	y		
FokI	GGATGNNNNNNNNN^NNNN_	BstPZ418I	y		
GsuI	CTGGAGNNNNNNNNNNNNNNN_NN^	-	y		
HgaI	GACGCNNNNN^NNNNN_	-	y		
HphI	GGTGANNNNNNN_N^	AsuHPI	y		
MboII	GAAGANNNNNNN_N^	-	y		
MlyI	GAGTCNNNNN^	SchI	y		
MmeI	TCCRACNNNNNNNNNNNNNNNNNN_NN^		-	-	
MnlI	CCTCNNNNNN_N^	-	y		
PleI	GAGTCNNNN^N_	PpsI	y		
RleAI	CCCACANNNNNNNNN_NNN^	-	-		
SfaNI	GCATCNNNNN^NNNN_	BspST5I	y		
SspD5I	GGTGANNNNNNNN^	-	-		
Sth132I	CCCGNNNN^NNNN_	-	-		
StsI	GGATGNNNNNNNNNN^NNNN_	-	-		
TaqII	GACCGANNNNNNNNN_NN^, CACCCANNNNNNNNN_NN^		-	-	
Tth111II	CAARCANNNNNNNNN_NN^	-	-		
UbaPI	CGAACG	-	-		

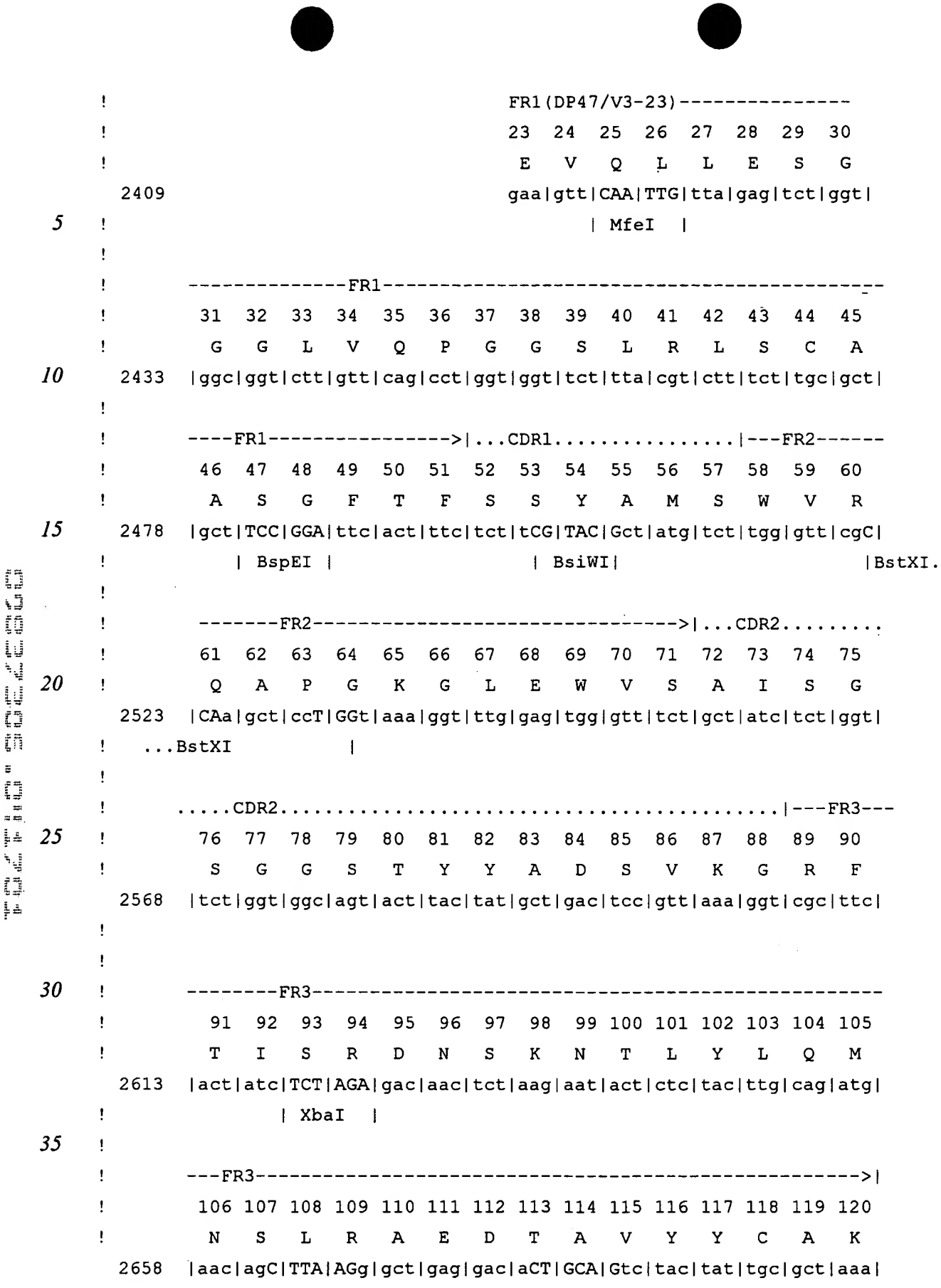
The notation is ^ means cut the upper strand and _ means cut the lower strand. If the upper and lower strand are cut at the same place, then only ^ appears.

Table 120: MALIA3, annotated

```
! MALIA3  9532 bases
!-----------------------------------------------------------------------
     1 aat gct act act att agt aga att gat gcc acc ttt tca gct cgc gcc
!    gene ii continued
    49 cca aat gaa aat ata gct aaa cag gtt att gac cat ttg cga aat gta
    97 tct aat ggt caa act aaa tct act cgt tcg cag aat tgg gaa tca act
   145 gtt aca tgg aat gaa act tcc aga cac cgt act tta gtt gca tat tta
   193 aaa cat gtt gag cta cag cac cag att cag caa tta agc tct aag cca
   241 tcc gca aaa atg acc tct tat caa aag gag caa tta aag gta ctc tct
   289 aat cct gac ctg ttg gag ttt gct tcc ggt ctg gtt cgc ttt gaa gct
   337 cga att aaa acg cga tat ttg aag tct ttc ggg ctt cct ctt aat ctt
   385 ttt gat gca atc cgc ttt gct tct gac tat aat agt cag ggt aaa gac
   433 ctg att ttt gat tta tgg tca ttc tcg ttt tct gaa ctg ttt aaa gca
   481 ttt gag ggg gat tca ATG aat att tat gac gat tcc gca gta ttg gac
!          RBS?......      Start gene x, ii continues
   529 gct atc cag tct aaa cat ttt act att acc ccc tct ggc aaa act tct
   577 ttt gca aaa gcc tct cgc tat ttt ggt ttt tat cgt cgt ctg gta aac
   625 gag ggt tat gat agt gtt gct ctt act atg cct cgt aat tcc ttt tgg
   673 cgt tat gta tct gca tta gtt gaa tgt ggt att cct aaa tct caa ctg
   721 atg aat ctt tct acc tgt aat aat gtt gtt ccg tta gtt cgt ttt att
   769 aac gta gat ttt tct tcc caa cgt cct gac tgg tat aat gag cca gtt
   817 ctt aaa atc gca TAA
!                    End X & II
   832 ggtaattca ca
!
!      M1              E5                  Q10                 T15
   843 ATG att aaa gtt gaa att aaa cca tct caa gcc caa ttt act act cgt
!     Start gene V
!
!     S17         S20                 P25                 E30
   891 tct ggt gtt tct cgt cag ggc aag cct tat tca ctg aat gag cag ctt
!
!             V35                 E40                 V45
   939 tgt tac gtt gat ttg ggt aat gaa tat ccg gtt ctt gtc aag att act
!
!          D50                 A55                 L60
   987 ctt gat gaa ggt cag cca gcc tat gcg cct ggt cTG TAC Acc gtt cat
!                                                   BsrGI...
```

```
!      L65                  V70                  S75                  R80
  1035 ctg tcc tct ttc aaa gtt ggt cag ttc ggt tcc ctt atg att gac cgt
!
!                       P85     K87 end of V
  1083 ctg cgc ctc gtt ccg gct aag TAA C
!
  1108 ATG gag cag gtc gcg gat ttc gac aca att tat cag gcg atg
!      Start gene VII
!
  1150 ata caa atc tcc gtt gta ctt tgt ttc gcg ctt ggt ata atc
!
!                         VII and IX overlap.
!                         ..... S2  V3  L4  V5                 S10
  1192 gct ggg ggt caa agA TGA gt gtt tta gtg tat tct ttc gcc tct ttc gtt
!                           End VII
!                         |start IX
!      L13     W15                 G20                 T25              E29
  1242 tta ggt tgg tgc ctt cgt agt ggc att acg tat ttt acc cgt tta atg gaa
!
  1293 act tcc tc
!
!       .... stop of IX, IX and VIII overlap by four bases
  1301 ATG aaa aag tct tta gtc ctc aaa gcc tct gta gcc gtt gct acc ctc
!      Start signal sequence of viii.
!
  1349 gtt ccg atg ctg tct ttc gct gct gag ggt gac gat ccc gca aaa gcg
!                                 mature VIII --->
  1397 gcc ttt aac tcc ctg caa gcc tca gcg acc gaa tat atc ggt tat gcg
  1445 tgg gcg atg gtt gtt gtc att
  1466 gtc ggc gca act atc ggt atc aag ctg ttt aag
  1499 aaa ttc acc tcg aaa gca ! 1515
!       ..........  -35  ..
!
  1517      agc tga taaaccgat acaattaaag gctccttttg
!                       ..... -10   ...
!
  1552 gagccttttt ttttGGAGAt ttt ! S.D. underlined
!
!           <------ III signal sequence ---------------------------->
```

```
!          M   K   K   L   L   F   A   I   P   L   V
1575 caac GTG aaa aaa tta tta ttc gca att cct tta gtt ! 1611
!
!      V   P   F   Y   S   H   S   A   Q
1612 gtt cct ttc tat tct cac aGT gcA Cag tCT
!                            ApaLI...
!
1642     GTC GTG ACG CAG CCG CCC TCA GTG TCT GGG GCC CCA GGG CAG
         AGG GTC ACC ATC TCC TGC ACT GGG AGC AGC TCC AAC ATC GGG GCA
!          BstEII...
1729     GGT TAT GAT GTA CAC TGG TAC CAG CAG CTT CCA GGA ACA GCC CCC AAA
1777     CTC CTC ATC TAT GGT AAC AGC AAT CGG CCC TCA GGG GTC CCT GAC CGA
1825     TTC TCT GGC TCC AAG TCT GGC ACC TCA GCC TCC CTG GCC ATC ACT
1870     GGG CTC CAG GCT GAG GAT GAG GCT GAT TAT
1900     TAC TGC CAG TCC TAT GAC AGC AGC CTG AGT
1930     GGC CTT TAT GTC TTC GGA ACT GGG ACC AAG GTC ACC GTC
!                                              BstEII...
1969     CTA GGT CAG CCC AAG GCC AAC CCC ACT GTC ACT
2002     CTG TTC CCG CCC TCC TCT GAG GAG CTC CAA GCC AAC AAG GCC ACA CTA
2050     GTG TGT CTG ATC AGT GAC TTC TAC CCG GGA GCT GTG ACA GTG GCC TGG
2098     AAG GCA GAT AGC AGC CCC GTC AAG GCG GGA GTG GAG ACC ACC ACA CCC
2146     TCC AAA CAA AGC AAC AAC AAG TAC GCG GCC AGC AGC TAT CTG AGC CTG
2194     ACG CCT GAG CAG TGG AAG TCC CAC AGA AGC TAC AGC TGC CAG GTC ACG
2242     CAT GAA GGG AGC ACC GTG GAG AAG ACA GTG GCC CCT ACA GAA TGT TCA
2290     TAA TAA ACCG CCTCCACCGG GCGCGCCAAT TCTATTTCAA GGAGACAGTC ATA
!                             AscI.....
!
!          PelB signal---------------------------------------------->
!           M   K   Y   L   L   P   T   A   A   A   G   L   L   L   L
2343     ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC
!
!          16  17  18  19  20      21  22
!          A   A   Q   P   A       M   A
2388     gcG GCC cag ccG GCC     atg gcc
!           SfiI.............
!                  NgoMI...(1/2)
!                         NcoI.........
!
```


FR1(DP47/V3-23)----------
23 24 25 26 27 28 29 30
E V Q L L E S G
2409
gaa|gtt|CAA|TTG|tta|gag|tct|ggt|
| MfeI |
----------FR1----------
31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
G G L V Q P G G S L R L S C A
2433 |ggc|ggt|ctt|gtt|cag|cct|ggt|ggt|tct|tta|cgt|ctt|tct|tgc|gct|
----FR1---------->|...CDR1.....|---FR2------
46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
A S G F T F S S Y A M S W V R
2478 |gct|TCC|GGA|ttc|act|ttc|tct|tCG|TAC|Gct|atg|tct|tgg|gtt|cgC|
| BspEI | | BsiWI| |BstXI.
-------FR2---------->|...CDR2.....
61 62 63 64 65 66 67 68 69 70 71 72 73 74 75
Q A P G K G L E W V S A I S G
2523 |CAa|gct|ccT|GGt|aaa|ggt|ttg|gag|tgg|gtt|tct|gct|atc|tct|ggt|
...BstXI |
.....CDR2.....|---FR3---
76 77 78 79 80 81 82 83 84 85 86 87 88 89 90
S G G S T Y Y A D S V K G R F
2568 |tct|ggt|ggc|agt|act|tac|tat|gct|gac|tcc|gtt|aaa|ggt|cgc|ttc|
--------FR3----------
91 92 93 94 95 96 97 98 99 100 101 102 103 104 105
T I S R D N S K N T L Y L Q M
2613 |act|atc|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|
| XbaI |
---FR3---------->|
106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
N S L R A E D T A V Y Y C A K
2658 |aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|tgc|gct|aaa|

```
!              |AflII |                | PstI |
!
!       .......CDR3.................|----FR4-------------------------
!        121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
!         D   Y   E   G   T   G   Y   A   F   D   I   W   G   Q   G
  2703  |gac|tat|gaa|ggt|act|ggt|tat|gct|ttc|gaC|ATA|TGg|ggt|caa|ggt|
!                                                | NdeI |(1/4)
!
!       --------------FR4---------->|
!        136 137 138 139 140 141 142
!         T   M   V   T   V   S   S
  2748  |act|atG|GTC|ACC|gtc|tct|agt
!              | BstEII |
! From BstEII onwards, pV323 is same as pCES1, except as noted.
! BstEII sites may occur in light chains; not likely to be unique in final
! vector.
!
!                          143 144 145 146 147 148 149 150 151 152
!                           A   S   T   K   G   P   S   V   F   P
  2769                     gcc tcc acc aaG GGC CCa tcg GTC TTC ccc
!                                       Bsp120I.      BbsI...(2/2)
!                                       ApaI....
!
!     153 154 155 156 157 158 159 160 161 162 163 164 165 166 167
!      L   A   P   S   S   K   S   T   S   G   G   T   A   A   L
  2799 ctg gca ccC TCC TCc aag agc acc tct ggg ggc aca gcg gcc ctg
!              BseRI...(2/2)
!
!       168 169 170 171 172 173 174 175 176 177 178 179 180 181 182
!        G   C   L   V   K   D   Y   F   P   E   P   V   T   V   S
  2844  ggc tgc ctg GTC AAG GAC TAC TTC CCc gaA CCG GTg acg gtg tcg
!                                           AgeI....
!
!       183 184 185 186 187 188 189 190 191 192 193 194 195 196 197
!        W   N   S   G   A   L   T   S   G   V   H   T   F   P   A
  2889  tgg aac tca GGC GCC ctg acc agc ggc gtc cac acc ttc ccg gct
!                  KasI...(1/4)
!
!       198 199 200 201 202 203 204 205 206 207 208 209 210 211 212
```

```
!          V   L   Q   S   S   G   L   Y   S   L   S   S   V   V   T
  2934   gtc cta cag tCt agc GGa ctc tac tcc ctc agc agc gta gtg acc
!                    (Bsu36I...)(knocked out)
!
5 !        213 214 215 216 217 218 219 220 221 222 223 224 225 226 227
!          V   P   S   S   S   L   G   T   Q   T   Y   I   C   N   V
  2979   gtg ccC tCt tct agc tTG Ggc acc cag acc tac atc tgc aac gtg
!                 (BstXI.....)N.B. destruction of BstXI & BpmI sites.
!
10 !       228 229 230 231 232 233 234 235 236 237 238 239 240 241 242
!          N   H   K   P   S   N   T   K   V   D   K   K   V   E   P
  3024   aat cac aag ccc agc aac acc aag gtg gac aag aaa gtt gag ccc
!
!          243 244 245
15 !       K   S   C   A   A   A   H   H   H   H   H   H   S   A
  3069   aaa tct tgt GCG GCC GCt cat cac cac cat cat cac tct gct
!                    NotI......
!
!          E   Q   K   L   I   S   E   E   D   L   N   G   A   A
20   3111   gaa caa aaa ctc atc tca gaa gag gat ctg aat ggt gcc gca
!
!
!          D   I   N   D   D   R   M     A   S   G   A
  3153   GAT ATC aac gat gat cgt atg   gct AGC  ggc gcc
25 !       rEK cleavage site.....   NheI...  KasI...
!          EcoRV..
!
! Domain 1 ------------------------------------------------------------
!            A   E   T   V   E   S   C   L   A
30   3183     gct gaa act gtt gaa agt tgt tta gca
!
!
!        K   P   H   T   E   I   S   F
  3210 aaa ccc cat aca gaa aat tca ttt
35 !
!        T   N   V   W   K   D   D   K   T
  3234 aCT AAC GTC TGG AAA GAC GAC AAA ACt
!
!        L   D   R   Y   A   N   Y   E   G   C   L   W   N   A   T   G   V
```

```
  3261 tta gat cgt tac gct aac tat gag ggt tgt ctg tgG AAT GCt aca ggc gtt
!                                                    BsmI____
!
!       V   V   C   T   G   D   E   T   Q   C   Y   G   T   W   V   P   I
  3312 gta gtt tgt act ggt GAC GAA ACT CAG TGT TAC GGT ACA TGG GTT cct att
!
!       G   L   A   I   P   E   N
  3363 ggg ctt gct atc cct gaa aat
!
! L1 linker ------------------------------------
!       E   G   G   G   S   E   G   G   G   S
  3384 gag ggt ggt ggc tct gag ggt ggc ggt tct
!
!       E   G   G   G   S   E   G   G   G   T
  3414 gag ggt ggc ggt tct gag ggt ggc ggt act
!
!  Domain 2 -----------------------------------
  3444 aaa cct cct gag tac ggt gat aca cct att ccg ggc tat act tat atc aac
  3495 cct ctc gac ggc act tat ccg cct ggt act gag caa aac ccc gct aat cct
  3546 aat cct tct ctt GAG GAG tct cag cct ctt aat act ttc atg ttt cag aat
!                     BseRI__
  3597 aat agg ttc cga aat agg cag ggg gca tta act gtt tat acg ggc act
  3645 gtt act caa ggc act gac ccc gtt aaa act tat tac cag tac act cct
  3693 gta tca tca aaa gcc atg tat gac gct tac tgg aac ggt aaa ttC AGA
!                                                                AlwNI
  3741 GAC TGc gct ttc cat tct ggc ttt aat gaa gat cca ttc gtt tgt gaa
!       AlwNI
  3789 tat caa ggc caa tcg tct gac ctg cct caa cct cct gtc aat gct
!
  3834 ggc ggc ggc tct
!  start L2 ----------------------------------------------------------
  3846 ggt ggt ggt tct
  3858 ggt ggc ggc tct
  3870 gag ggt ggt ggc tct gag ggt ggc ggt tct
  3900 gag ggt ggc ggc tct gag gga ggc ggt tcc
  3930 ggt ggt ggc tct ggt    ! end L2
!
! Domain 3 ----------------------------------------------------------
!       S   G   D   F   D   Y   E   K   M   A   N   A   N   K   G   A
```

```
  3945 tcc ggt gat ttt gat tat gaa aag atg gca aac gct aat aag ggg gct
!
!       M   T   E   N   A   D   E   N   A   L   Q   S   D   A   K   G
  3993 atg acc gaa aat gcc gat gaa aac gcg cta cag tct gac gct aaa ggc
!
!       K   L   D   S   V   A   T   D   Y   G   A   A   I   D   G   F
  4041 aaa ctt gat tct gtc gct act gat tac ggt gct gct atc gat ggt ttc
!
!       I   G   D   V   S   G   L   A   N   G   N   G   A   T   G   D
  4089 att ggt gac gtt tcc ggc ctt gct aat ggt aat ggt gct act ggt gat
!
!       F   A   G   S   N   S   Q   M   A   Q   V   G   D   G   D   N
  4137 ttt gct ggc tct aat tcc caa atg gct caa gtc ggt gac ggt gat aat
!
!       S   P   L   M   N   N   F   R   Q   Y   L   P   S   L   P   Q
  4185 tca cct tta atg aat aat ttc cgt caa tat tta cct tcc ctc cct caa
!
!       S   V   E   C   R   P   F   V   F   S   A   G   K   P   Y   E
  4233 tcg gtt gaa tgt cgc cct ttt gtc ttt agc gct ggt aaa cca tat gaa
!
!       F   S   I   D   C   D   K   I   N   L   F   R
  4281 ttt tct att gat tgt gac aaa ata aac tta ttc cgt
!                                                   End Domain 3
!
!       G   V   F   A   F   L   L   Y   V   A   T   F   M   Y   V  F140
  4317 ggt gtc ttt gcg ttt ctt tta tat gtt gcc acc ttt atg tat gta ttt
!      start transmembrane segment
!
!       S   T   F   A   N   I   L
  4365 tct acg ttt gct aac ata ctg
!
!       R   N   K   E   S
  4386 cgt aat aag gag tct TAA ! stop of iii
!     Intracellular anchor.
!
!           M1  P2  V   L  L5   G   I   P   L  L10  L   R   F   L  G15
  4404  tc ATG cca gtt ctt ttg ggt att ccg tta tta ttg cgt ttc ctc ggt
!          Start VI
!
```

```
4451 ttc ctt ctg gta act ttg ttc ggc tat ctg ctt act ttt ctt aaa aag
4499 ggc ttc ggt aag ata gct att gct att tca ttg ttt ctt gct ctt att
4547 att ggg ctt aac tca att ctt gtg ggt tat ctc tct gat att agc gct
4595 caa tta ccc tct gac ttt gtt cag ggt gtt cag tta att ctc ccg tct
4643 aat gcg ctt ccc tgt ttt tat gtt att ctc tct gta aag gct gct att
4691 ttc att ttt gac gtt aaa caa aaa atc gtt tct tat ttg gat tgg gat
!
!                M1  A2  V3      F5                  L10         G13
4739 aaa TAA t ATG gct gtt tat ttt gta act ggc aaa tta ggc tct gga
!       end VI   Start gene I
!
!       14  15  16  17  18  19  20  21  22  23  24  25  26  27  28
!       K   T   L   V   S   V   G   K   I   Q   D   K   I   V   A
4785 aag acg ctc gtt agc gtt ggt aag att cag gat aaa att gta gct
!
!       29  30  31  32  33  34  35  36  37  38  39  40  41  42  43
!       G   C   K   I   A   T   N   L   D   L   R   L   Q   N   L
4830 ggg tgc aaa ata gca act aat ctt gat tta agg ctt caa aac ctc
!
!       44  45  46  47  48  49  50  51  52  53  54  55  56  57  58
!       P   Q   V   G   R   F   A   K   T   P   R   V   L   R   I
4875 ccg caa gtc ggg agg ttc gct aaa acg cct cgc gtt ctt aga ata
!
!       59  60  61  62  63  64  65  66  67  68  69  70  71  72  73
!       P   D   K   P   S   I   S   D   L   L   A   I   G   R   G
4920 ccg gat aag cct tct ata tct gat ttg ctt gct att ggg cgc ggt
!
!       74  75  76  77  78  79  80  81  82  83  84  85  86  87  88
!       N   D   S   Y   D   E   N   K   N   G   L   L   V   L   D
4965 aat gat tcc tac gat gaa aat aaa aac ggc ttg ctt gtt ctc gat
!
!       89  90  91  92  93  94  95  96  97  98  99 100 101 102 103
!       E   C   G   T   W   F   N   T   R   S   W   N   D   K   E
5010 gag tgc ggt act tgg ttt aat acc cgt tct tgg aat gat aag gaa
!
!      104 105 106 107 108 109 110 111 112 113 114 115 116 117 118
!       R   Q   P   I   I   D   W   F   L   H   A   R   K   L   G
5055 aga cag ccg att att gat tgg ttt cta cat gct cgt aaa tta gga
!
```

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!      119 120 121 122 123 124 125 126 127 128 129 130 131 132 133
!       W   D   I   I   F   L   V   Q   D   L   S   I   V   D   K
  5100 tgg gat att att ttt ctt gtt cag gac tta tct att gtt gat aaa
!
!      134 135 136 137 138 139 140 141 142 143 144 145 146 147 148
!       Q   A   R   S   A   L   A   E   H   V   V   Y   C   R   R
  5145 cag gcg cgt tct gca tta gct gaa cat gtt gtt tat tgt cgt cgt
!
!      149 150 151 152 153 154 155 156 157 158 159 160 161 162 163
!       L   D   R   I   T   L   P   F   V   G   T   L   Y   S   L
  5190 ctg gac aga att act tta cct ttt gtc ggt act tta tat tct ctt
!
!      164 165 166 167 168 169 170 171 172 173 174 175 176 177 178
!       I   T   G   S   K   M   P   L   P   K   L   H   V   G   V
  5235 att act ggc tcg aaa atg cct ctg cct aaa tta cat gtt ggc gtt
!
!      179 180 181 182 183 184 185 186 187 188 189 190 191 192 193
!       V   K   Y   G   D   S   Q   L   S   P   T   V   E   R   W
  5280 gtt aaa tat ggc gat tct caa tta agc cct act gtt gag cgt tgg
!
!      194 195 196 197 198 199 200 201 202 203 204 205 206 207 208
!       L   Y   T   G   K   N   L   Y   N   A   Y   D   T   K   Q
  5325 ctt tat act ggt aag aat ttg tat aac gca tat gat act aaa cag
!
!      209 210 211 212 213 214 215 216 217 218 219 220 221 222 223
!       A   F   S   S   N   Y   D   S   G   V   Y   S   Y   L   T
  5370 gct ttt tct agt aat tat gat tcc ggt gtt tat tct tat tta acg
!
!      224 225 226 227 228 229 230 231 232 233 234 235 236 237 238
!       P   Y   L   S   H   G   R   Y   F   K   P   L   N   L   G
  5415 cct tat tta tca cac ggt cgg tat ttc aaa cca tta aat tta ggt
!
!      239 240 241 242 243 244 245 246 247 248 249 250 251 252 253
!       Q   K   M   K   L   T   K   I   Y   L   K   K   F   S   R
  5460 cag aag atg aaa tta act aaa ata tat ttg aaa aag ttt tct cgc
!
!      254 255 256 257 258 259 260 261 262 263 264 265 266 267 268
!       V   L   C   L   A   I   G   F   A   S   A   F   T   Y   S
  5505 gtt ctt tgt ctt gcg att gga ttt gca tca gca ttt aca tat agt
```

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!
!      269 270 271 272 273 274 275 276 277 278 279 280 281 282 283
!       Y   I   T   Q   P   K   P   E   V   K   K   V   V   S   Q
  5550 tat ata acc caa cct aag ccg gag gtt aaa aag gta gtc tct cag
!
!      284 285 286 287 288 289 290 291 292 293 294 295 296 297 298
!       T   Y   D   F   D   K   F   T   I   D   S   S   Q   R   L
  5595 acc tat gat ttt gat aaa ttc act att gac tct tct cag cgt ctt
!
!      299 300 301 302 303 304 305 306 307 308 309 310 311 312 313
!       N   L   S   Y   R   Y   V   F   K   D   S   K   G   K   L
  5640 aat cta agc tat cgc tat gtt ttc aag gat tct aag gga aaa TTA
!                                                              PacI
!
!      314 315 316 317 318 319 320 321 322 323 324 325 326 327 328
!       I   N   S   D   D   L   Q   K   Q   G   Y   S   L   T   Y
  5685 ATT AAt agc gac gat tta cag aag caa ggt tat tca ctc aca tat
!     PacI
!
!      329 330 331 332 333 334 335 336 337 338 339 340 341 342 343
!      i  I   D   L   C   T   V   S   I   K   K   G   N   S   N   E
!     iv                                                        M1  K
  5730   att gat tta tgt act gtt tcc att aaa aaa ggt aat tca aAT Gaa
!                                                             Start IV
!
!         344 345 346 347 348 349
!     i    I   V   K   C   N   .End of I
!     iv    L3  L   N5  V   I7  N    F  V10
  5775    att gtt aaa tgt aat TAA T TTT GTT
!  IV continued.....
  5800 ttc ttg atg ttt gtt tca tca tct tct ttt gct cag gta att gaa atg
  5848 aat aat tcg cct ctg cgc gat ttt gta act tgg tat tca aag caa tca
  5896 ggc gaa tcc gtt att gtt tct ccc gat gta aaa ggt act gtt act gta
  5944 tat tca tct gac gtt aaa cct gaa aat cta cgc aat ttc ttt att tct
  5992 gtt tta cgt gct aat aat ttt gat atg gtt ggt tca att cct tcc ata
  6040 att cag aag tat aat cca aac aat cag gat tat att gat gaa ttg cca
  6088 tca tct gat aat cag gaa tat gat gat aat tcc gct cct tct ggt ggt
  6136 ttc ttt gtt ccg caa aat gat aat gtt act caa act ttt aaa att aat
  6184 aac gtt cgg gca aag gat tta ata cga gtt gtc gaa ttg ttt gta aag
```

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  6232 tct aat act tct aaa tcc tca aat gta tta tct att gac ggc tct aat
  6280 cta tta gtt gtt TCT gca cct aaa gat att tta gat aac ctt cct caa
!                     ApaLI removed
  6328 ttc ctt tct act gtt gat ttg cca act gac cag ata ttg att gag ggt
  6376 ttg ata ttt gag gtt cag caa ggt gat gct tta gat ttt tca ttt gct
  6424 gct ggc tct cag cgt ggc act gtt gca ggc ggt gtt aat act gac cgc
  6472 ctc acc tct gtt tta tct tct gct ggt ggt tcg ttc ggt att ttt aat
  6520 ggc gat gtt tta ggg cta tca gtt cgc gca tta aag act aat agc cat
  6568 tca aaa ata ttg tct gtg cca cgt att ctt acg ctt tca ggt cag aag
  6616 ggt tct atc tct gtT GGC CAg aat gtc cct ttt att act ggt cgt gtg
!                     MscI____
  6664 act ggt gaa tct gcc aat gta aat aat cca ttt cag acg att gag cgt
  6712 caa aat gta ggt att tcc atg agc gtt ttt cct gtt gca atg gct ggc
  6760 ggt aat att gtt ctg gat att acc agc aag gcc gat agt ttg agt tct
  6808 tct act cag gca agt gat gtt att act aat caa aga agt att gct aca
  6856 acg gtt aat ttg cgt gat gga cag act ctt tta ctc ggt ggc ctc act
  6904 gat tat aaa aac act tct caa gat tct ggc gta ccg ttc ctg tct aaa
  6952 atc cct tta atc ggc ctc ctg ttt agc tcc cgc tct gat tcc aac gag
  7000 gaa agc acg tta tac gtg ctc gtc aaa gca acc ata gta cgc gcc ctg
  7048 TAG cggcgcatt
!     End IV
  7060 aagcgcggcg ggtgtggtgg ttacgcgcag cgtgaccgct acacttgcca gcgccctagc
  7120 gcccgctcct ttcgctttct tcccttcctt tctcgccacg ttcGCCGGCt ttccccgtca
!                                                  NgoMI_
  7180 agctctaaat cgggggctcc ctttagggtt ccgatttagt gctttacggc acctcgaccc
  7240 caaaaaactt gatttgggtg atggttCACG TAGTGggcca tcgccctgat agacggtttt
!                                  DraIII____
  7300 tcgcccttTG ACGTTGGAGT Ccacgttctt taatagtgga ctcttgttcc aaactggaac
!              DrdI__________
  7360 aacactcaac cctatctcgg gctattcttt tgatttataa gggattttgc cgatttcgga
  7420 accaccatca aacaggattt tcgcctgctg gggcaaacca gcgtggaccg cttgctgcaa
  7480 ctctctcagg gccaggcggt gaagggcaat CAGCTGttgc cCGTCTCact ggtgaaaaga
!                                     PvuII.      BsmBI.
  7540 aaaaccaccc tGGATCC  AAGCTT
!                BamHI   HindIII (1/2)
!                Insert carrying bla gene
  7563    gcaggtg gcacttttcg gggaaatgtg cgcggaaccc
  7600 ctatttgttt atttttctaa atacattcaa atatGTATCC gctcatgaga caataaccct
!                                        BciVI
```

```
  7660 gataaatgct tcaataatat tgaaaaAGGA AGAgt
!                                  RBS.?...
!      Start bla gene
  7695 ATG agt att caa cat ttc cgt gtc gcc ctt att ccc ttt ttt gcg gca ttt
  7746 tgc ctt cct gtt ttt gct cac cca gaa acg ctg gtg aaa gta aaa gat gct
  7797 gaa gat cag ttg ggC gCA CGA Gtg ggt tac atc gaa ctg gat ctc aac agc
!                           BssSI...
!                       ApaLI removed
  7848 ggt aag atc ctt gag agt ttt cgc ccc gaa gaa cgt ttt cca atg atg agc
  7899 act ttt aaa gtt ctg cta tgt cat aca cta tta tcc cgt att gac gcc ggg
  7950 caa gaG CAA CTC GGT CGc cgg gcg cgg tat tct cag aat gac ttg gtt gAG
!            BcgI_____________                                        ScaI
  8001 TAC Tca cca gtc aca gaa aag cat ctt acg gat ggc atg aca gta aga gaa
!      ScaI_
  8052 tta tgc agt gct gcc ata acc atg agt gat aac act gcg gcc aac tta ctt
  8103 ctg aca aCG ATC Gga gga ccg aag gag cta acc gct ttt ttg cac aac atg
!               PvuI____
  8154 ggg gat cat gta act cgc ctt gat cgt tgg gaa ccg gag ctg aat gaa gcc
  8205 ata cca aac gac gag cgt gac acc acg atg cct gta gca atg cca aca acg
  8256 tTG CGC Aaa cta tta act ggc gaa cta ctt act cta gct tcc cgg caa caa
!       FspI....
!
  8307 tta ata gac tgg atg gag gcg gat aaa gtt gca gga cca ctt ctg cgc tcg
  8358 GCC ctt ccG GCt ggc tgg ttt att gct gat aaa tct gga gcc ggt gag cgt
!      BglI__________
  8409 gGG TCT Cgc ggt atc att gca gca ctg ggg cca gat ggt aag ccc tcc cgt
!       BsaI____
  8460 atc gta gtt atc tac acG ACg ggg aGT Cag gca act atg gat gaa cga aat
!                            AhdI___________
  8511 aga cag atc gct gag ata ggt gcc tca ctg att aag cat tgg TAA ctgt
!                                                              stop
  8560 cagaccaagt ttactcatat atactttaga ttgatttaaa acttcatttt taatttaaaa
  8620 ggatctaggt gaagatcctt tttgataatc tcatgaccaa aatcccttaa cgtgagtttt
  8680 cgttccactg tacgtaagac cccc
  8704 AAGCTT   GTCGAC tgaa tggcgaatgg cgctttgcct
!      HindIII  SalI..
!      (2/2)    HincII
  8740 ggtttccggc accagaagcg gtgccggaaa gctggctgga gtgcgatctt
!
```

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  8790 CCTGAGG
!      Bsu36I_
  8797      ccgat actgtcgtcg tcccctcaaa ctggcagatg
  8832 cacggttacg atgcgcccat ctacaccaac gtaacctatc ccattacggt caatccgccg
  8892 tttgttccca cggagaatcc gacgggttgt tactcgctca catttaatgt tgatgaaagc
  8952 tggctacagg aaggccagac gcgaattatt tttgatggcg ttcctattgg ttaaaaaatg
  9012 agctgattta acaaaaattt aacgcgaatt ttaacaaaat attaacgttt acaATTTAAA
!                                                                SwaI...
  9072 Tatttgctta tacaatcttc ctgtttttgg ggcttttctg attatcaacc GGGGTAcat
!                                                              RBS?
  9131 ATG att gac atg cta gtt tta cga tta ccg ttc atc gat tct ctt gtt tgc
!      Start gene II
  9182 tcc aga ctc tca ggc aat gac ctg ata gcc ttt gtA GAT CTc tca aaa ata
!                                                   BglII...
  9233 gct acc ctc tcc ggc atg aat tta tca gct aga acg gtt gaa tat cat att
  9284 gat ggt gat ttg act gtc tcc ggc ctt tct cac cct ttt gaa tct tta cct
  9335 aca cat tac tca ggc att gca ttt aaa ata tat gag ggt tct aaa aat ttt
  9386 tat cct tgc gtt gaa ata aag gct tct ccc gca aaa gta tta cag ggt cat
  9437 aat gtt ttt ggt aca acc gat tta gct tta tgc tct gag gct tta ttg ctt
  9488 aat ttt gct aat tct ttg cct tgc ctg tat gat tta ttg gat gtt ! 9532
! gene II continues
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Table 120B: Sequence of MALIA3, condensed

LOCUS MALIA3 9532 CIRCULAR

ORIGIN

```
       1 AATGCTACTA CTATTAGTAG AATTGATGCC ACCTTTTCAG CTCGCGCCCC AAATGAAAAT
      61 ATAGCTAAAC AGGTTATTGA CCATTTGCGA AATGTATCTA ATGGTCAAAC TAAATCTACT
     121 CGTTCGCAGA ATTGGGAATC AACTGTTACA TGGAATGAAA CTTCCAGACA CCGTACTTTA
     181 GTTGCATATT TAAAACATGT TGAGCTACAG CACCAGATTC AGCAATTAAG CTCTAAGCCA
     241 TCCGCAAAAA TGACCTCTTA TCAAAAGGAG CAATTAAAGG TACTCTCTAA TCCTGACCTG
     301 TTGGAGTTTG CTTCCGGTCT GGTTCGCTTT GAAGCTCGAA TTAAAACGCG ATATTTGAAG
     361 TCTTTCGGGC TTCCTCTTAA TCTTTTTGAT GCAATCCGCT TTGCTTCTGA CTATAATAGT
     421 CAGGGTAAAG ACCTGATTTT TGATTTATGG TCATTCTCGT TTTCTGAACT GTTTAAAGCA
     481 TTTGAGGGGG ATTCAATGAA TATTTATGAC GATTCCGCAG TATTGGACGC TATCCAGTCT
     541 AAACATTTTA CTATTACCCC CTCTGGCAAA ACTTCTTTTG CAAAAGCCTC TCGCTATTTT
     601 GGTTTTTATC GTCGTCTGGT AAACGAGGGT TATGATAGTG TTGCTCTTAC TATGCCTCGT
     661 AATTCCTTTT GGCGTTATGT ATCTGCATTA GTTGAATGTG GTATTCCTAA ATCTCAACTG
     721 ATGAATCTTT CTACCTGTAA TAATGTTGTT CCGTTAGTTC GTTTTATTAA CGTAGATTTT
     781 TCTTCCCAAC GTCCTGACTG GTATAATGAG CCAGTTCTTA AAATCGCATA AGGTAATTCA
     841 CAATGATTAA AGTTGAAATT AAACCATCTC AAGCCCAATT TACTACTCGT TCTGGTGTTT
     901 CTCGTCAGGG CAAGCCTTAT TCACTGAATG AGCAGCTTTG TTACGTTGAT TTGGGTAATG
     961 AATATCCGGT TCTTGTCAAG ATTACTCTTG ATGAAGGTCA GCCAGCCTAT GCGCCTGGTC
    1021 TGTACACCGT TCATCTGTCC TCTTTCAAAG TTGGTCAGTT CGGTTCCCTT ATGATTGACC
    1081 GTCTGCGCCT CGTTCCGGCT AAGTAACATG GAGCAGGTCG CGGATTTCGA CACAATTTAT
    1141 CAGGCGATGA TACAAATCTC CGTTGTACTT TGTTTCGCGC TTGGTATAAT CGCTGGGGGT
    1201 CAAAGATGAG TGTTTTAGTG TATTCTTTCG CCTCTTTCGT TTTAGGTTGG TGCCTTCGTA
    1261 GTGGCATTAC GTATTTTACC CGTTTAATGG AAACTTCCTC ATGAAAAAGT CTTTAGTCCT
    1321 CAAAGCCTCT GTAGCCGTTG CTACCCTCGT TCCGATGCTG TCTTTCGCTG CTGAGGGTGA
    1381 CGATCCCGCA AAAGCGGCCT TTAACTCCCT GCAAGCCTCA GCGACCGAAT ATATCGGTTA
    1441 TGCGTGGGCG ATGGTTGTTG TCATTGTCGG CGCAACTATC GGTATCAAGC TGTTTAAGAA
    1501 ATTCACCTCG AAAGCAAGCT GATAAACCGA TACAATTAAA GGCTCCTTTT GGAGCCTTTT
    1561 TTTTTGGAGA TTTTCAACGT GAAAAAATTA TTATTCGCAA TTCCTTTAGT TGTTCCTTTC
    1621 TATTCTCACA GTGCACAGTC TGTCGTGACG CAGCCGCCCT CAGTGTCTGG GGCCCCAGGG
    1681 CAGAGGGTCA CCATCTCCTG CACTGGGAGC AGCTCCAACA TCGGGGCAGG TTATGATGTA
    1741 CACTGGTACC AGCAGCTTCC AGGAACAGCC CCCAAACTCC TCATCTATGG TAACAGCAAT
    1801 CGGCCCTCAG GGGTCCCTGA CCGATTCTCT GGCTCCAAGT CTGGCACCTC AGCCTCCCTG
    1861 GCCATCACTG GGCTCCAGGC TGAGGATGAG GCTGATTATT ACTGCCAGTC CTATGACAGC
    1921 AGCCTGAGTG GCCTTTATGT CTTCGGAACT GGGACCAAGG TCACCGTCCT AGGTCAGCCC
    1981 AAGGCCAACC CCACTGTCAC TCTGTTCCCG CCCTCCTCTG AGGAGCTCCA AGCCAACAAG
    2041 GCCACACTAG TGTGTCTGAT CAGTGACTTC TACCCGGGAG CTGTGACAGT GGCCTGGAAG
    2101 GCAGATAGCA GCCCCGTCAA GGCGGGAGTG GAGACCACCA CACCCTCCAA ACAAAGCAAC
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2161 AACAAGTACG CGGCCAGCAG CTATCTGAGC CTGACGCCTG AGCAGTGGAA GTCCCACAGA
2221 AGCTACAGCT GCCAGGTCAC GCATGAAGGG AGCACCGTGG AGAAGACAGT GGCCCCTACA
2281 GAATGTTCAT AATAAACCGC CTCCACCGGG CGCGCCAATT CTATTTCAAG GAGACAGTCA
2341 TAATGAAATA CCTATTGCCT ACGGCAGCCG CTGGATTGTT ATTACTCGCG GCCCAGCCGG
2401 CCATGGCCGA AGTTCAATTG TTAGAGTCTG GTGGCGGTCT TGTTCAGCCT GGTGGTTCTT
2461 TACGTCTTTC TTGCGCTGCT TCCGGATTCA CTTTCTCTTC GTACGCTATG TCTTGGGTTC
2521 GCCAAGCTCC TGGTAAAGGT TTGGAGTGGG TTTCTGCTAT CTCTGGTTCT GGTGGCAGTA
2581 CTTACTATGC TGACTCCGTT AAAGGTCGCT TCACTATCTC TAGAGACAAC TCTAAGAATA
2641 CTCTCTACTT GCAGATGAAC AGCTTAAGGG CTGAGGACAC TGCAGTCTAC TATTGCGCTA
2701 AAGACTATGA AGGTACTGGT TATGCTTTCG ACATATGGGG TCAAGGTACT ATGGTCACCG
2761 TCTCTAGTGC CTCCACCAAG GGCCCATCGG TCTTCCCCCT GGCACCCTCC TCCAAGAGCA
2821 CCTCTGGGGG CACAGCGGCC CTGGGCTGCC TGGTCAAGGA CTACTTCCCC GAACCGGTGA
2881 CGGTGTCGTG GAACTCAGGC GCCCTGACCA GCGGCGTCCA CACCTTCCCG GCTGTCCTAC
2941 AGTCTAGCGG ACTCTACTCC CTCAGCAGCG TAGTGACCGT GCCCTCTTCT AGCTTGGGCA
3001 CCCAGACCTA CATCTGCAAC GTGAATCACA AGCCCAGCAA CACCAAGGTG GACAAGAAAG
3061 TTGAGCCCAA ATCTTGTGCG GCCGCTCATC ACCACCATCA TCACTCTGCT GAACAAAAAC
3121 TCATCTCAGA AGAGGATCTG AATGGTGCCG CAGATATCAA CGATGATCGT ATGGCTGGCG
3181 CCGCTGAAAC TGTTGAAAGT TGTTTAGCAA AACCCCATAC AGAAAATTCA TTTACTAACG
3241 TCTGGAAAGA CGACAAAACT TTAGATCGTT ACGCTAACTA TGAGGGTTGT CTGTGGAATG
3301 CTACAGGCGT TGTAGTTTGT ACTGGTGACG AAACTCAGTG TTACGGTACA TGGGTTCCTA
3361 TTGGGCTTGC TATCCCTGAA AATGAGGGTG GTGGCTCTGA GGGTGGCGGT TCTGAGGGTG
3421 GCGGTTCTGA GGGTGGCGGT ACTAAACCTC CTGAGTACGG TGATACACCT ATTCCGGGCT
3481 ATACTTATAT CAACCCTCTC GACGGCACTT ATCCGCCTGG TACTGAGCAA AACCCCGCTA
3541 ATCCTAATCC TTCTCTTGAG GAGTCTCAGC CTCTTAATAC TTTCATGTTT CAGAATAATA
3601 GGTTCCGAAA TAGGCAGGGG GCATTAACTG TTTATACGGG CACTGTTACT CAAGGCACTG
3661 ACCCCGTTAA AACTTATTAC CAGTACACTC CTGTATCATC AAAAGCCATG TATGACGCTT
3721 ACTGGAACGG TAAATTCAGA GACTGCGCTT TCCATTCTGG CTTTAATGAA GATCCATTCG
3781 TTTGTGAATA TCAAGGCCAA TCGTCTGACC TGCCTCAACC TCCTGTCAAT GCTGGCGGCG
3841 GCTCTGGTGG TGGTTCTGGT GGCGGCTCTG AGGGTGGTGG CTCTGAGGGT GGCGGTTCTG
3901 AGGGTGGCGG CTCTGAGGGA GGCGGTTCCG GTGGTGGCTC TGGTTCCGGT GATTTTGATT
3961 ATGAAAAGAT GGCAAACGCT AATAAGGGGG CTATGACCGA AAATGCCGAT GAAAACGCGC
4021 TACAGTCTGA CGCTAAAGGC AAACTTGATT CTGTCGCTAC TGATTACGGT GCTGCTATCG
4081 ATGGTTTCAT TGGTGACGTT TCCGGCCTTG CTAATGGTAA TGGTGCTACT GGTGATTTTG
4141 CTGGCTCTAA TTCCCAAATG GCTCAAGTCG GTGACGGTGA TAATTCACCT TTAATGAATA
4201 ATTTCCGTCA ATATTTACCT TCCCTCCCTC AATCGGTTGA ATGTCGCCCT TTTGTCTTTA
4261 GCGCTGGTAA ACCATATGAA TTTTCTATTG ATTGTGACAA AATAAACTTA TTCCGTGGTG
4321 TCTTTGCGTT TCTTTTATAT GTTGCCACCT TTATGTATGT ATTTTCTACG TTTGCTAACA
4381 TACTGCGTAA TAAGGAGTCT TAATCATGCC AGTTCTTTTG GGTATTCCGT TATTATTGCG
4441 TTTCCTCGGT TTCCTTCTGG TAACTTTGTT CGGCTATCTG CTTACTTTTC TTAAAAAGGG

4501 CTTCGGTAAG ATAGCTATTG CTATTTCATT GTTTCTTGCT CTTATTATTG GGCTTAACTC
4561 AATTCTTGTG GGTTATCTCT CTGATATTAG CGCTCAATTA CCCTCTGACT TTGTTCAGGG
4621 TGTTCAGTTA ATTCTCCCGT CTAATGCGCT TCCCTGTTTT TATGTTATTC TCTCTGTAAA
4681 GGCTGCTATT TTCATTTTTG ACGTTAAACA AAAAATCGTT TCTTATTTGG ATTGGGATAA
4741 ATAATATGGC TGTTTATTTT GTAACTGGCA AATTAGGCTC TGGAAAGACG CTCGTTAGCG
4801 TTGGTAAGAT TCAGGATAAA ATTGTAGCTG GGTGCAAAAT AGCAACTAAT CTTGATTTAA
4861 GGCTTCAAAA CCTCCCGCAA GTCGGGAGGT TCGCTAAAAC GCCTCGCGTT CTTAGAATAC
4921 CGGATAAGCC TTCTATATCT GATTTGCTTG CTATTGGGCG CGGTAATGAT TCCTACGATG
4981 AAAATAAAAA CGGCTTGCTT GTTCTCGATG AGTGCGGTAC TTGGTTTAAT ACCCGTTCTT
5041 GGAATGATAA GGAAAGACAG CCGATTATTG ATTGGTTTCT ACATGCTCGT AAATTAGGAT
5101 GGGATATTAT TTTTCTTGTT CAGGACTTAT CTATTGTTGA TAAACAGGCG CGTTCTGCAT
5161 TAGCTGAACA TGTTGTTTAT TGTCGTCGTC TGGACAGAAT TACTTTACCT TTTGTCGGTA
5221 CTTTATATTC TCTTATTACT GGCTCGAAAA TGCCTCTGCC TAAATTACAT GTTGGCGTTG
5281 TTAAATATGG CGATTCTCAA TTAAGCCCTA CTGTTGAGCG TTGGCTTTAT ACTGGTAAGA
5341 ATTTGTATAA CGCATATGAT ACTAAACAGG CTTTTTCTAG TAATTATGAT TCCGGTGTTT
5401 ATTCTTATTT AACGCCTTAT TTATCACACG GTCGGTATTT CAAACCATTA AATTTAGGTC
5461 AGAAGATGAA ATTAACTAAA ATATATTTGA AAAAGTTTTC TCGCGTTCTT TGTCTTGCGA
5521 TTGGATTTGC ATCAGCATTT ACATATAGTT ATATAACCCA ACCTAAGCCG GAGGTTAAAA
5581 AGGTAGTCTC TCAGACCTAT GATTTTGATA AATTCACTAT TGACTCTTCT CAGCGTCTTA
5641 ATCTAAGCTA TCGCTATGTT TTCAAGGATT CTAAGGGAAA ATTAATTAAT AGCGACGATT
5701 TACAGAAGCA AGGTTATTCA CTCACATATA TTGATTTATG TACTGTTTCC ATTAAAAAAG
5761 GTAATTCAAA TGAAATTGTT AAATGTAATT AATTTTGTTT TCTTGATGTT TGTTTCATCA
5821 TCTTCTTTTG CTCAGGTAAT TGAAATGAAT AATTCGCCTC TGCGCGATTT TGTAACTTGG
5881 TATTCAAAGC AATCAGGCGA ATCCGTTATT GTTTCTCCCG ATGTAAAAGG TACTGTTACT
5941 GTATATTCAT CTGACGTTAA ACCTGAAAAT CTACGCAATT TCTTTATTTC TGTTTTACGT
6001 GCTAATAATT TTGATATGGT TGGTTCAATT CCTTCCATAA TTCAGAAGTA TAATCCAAAC
6061 AATCAGGATT ATATTGATGA ATTGCCATCA TCTGATAATC AGGAATATGA TGATAATTCC
6121 GCTCCTTCTG GTGGTTTCTT TGTTCCGCAA AATGATAATG TTACTCAAAC TTTTAAAATT
6181 AATAACGTTC GGGCAAAGGA TTTAATACGA GTTGTCGAAT TGTTTGTAAA GTCTAATACT
6241 TCTAAATCCT CAAATGTATT ATCTATTGAC GGCTCTAATC TATTAGTTGT TTCTGCACCT
6301 AAAGATATTT TAGATAACCT TCCTCAATTC CTTTCTACTG TTGATTTGCC AACTGACCAG
6361 ATATTGATTG AGGGTTTGAT ATTTGAGGTT CAGCAAGGTG ATGCTTTAGA TTTTTCATTT
6421 GCTGCTGGCT CTCAGCGTGG CACTGTTGCA GGCGGTGTTA ATACTGACCG CCTCACCTCT
6481 GTTTTATCTT CTGCTGGTGG TTCGTTCGGT ATTTTTAATG GCGATGTTTT AGGGCTATCA
6541 GTTCGCGCAT TAAAGACTAA TAGCCATTCA AAAATATTGT CTGTGCCACG TATTCTTACG
6601 CTTTCAGGTC AGAAGGGTTC TATCTCTGTT GGCCAGAATG TCCCTTTTAT TACTGGTCGT
6661 GTGACTGGTG AATCTGCCAA TGTAAATAAT CCATTTCAGA CGATTGAGCG TCAAAATGTA
6721 GGTATTTCCA TGAGCGTTTT TCCTGTTGCA ATGGCTGGCG GTAATATTGT TCTGGATATT
6781 ACCAGCAAGG CCGATAGTTT GAGTTCTTCT ACTCAGGCAA GTGATGTTAT TACTAATCAA

6841 AGAAGTATTG CTACAACGGT TAATTTGCGT GATGGACAGA CTCTTTTACT CGGTGGCCTC
6901 ACTGATTATA AAAACACTTC TCAAGATTCT GGCGTACCGT TCCTGTCTAA AATCCCTTTA
6961 ATCGGCCTCC TGTTTAGCTC CCGCTCTGAT TCCAACGAGG AAAGCACGTT ATACGTGCTC
7021 GTCAAAGCAA CCATAGTACG CGCCCTGTAG CGGCGCATTA AGCGCGGCGG GTGTGGTGGT
7081 TACGCGCAGC GTGACCGCTA CACTTGCCAG CGCCCTAGCG CCCGCTCCTT TCGCTTTCTT
7141 CCCTTCCTTT CTCGCCACGT TCGCCGGCTT TCCCCGTCAA GCTCTAAATC GGGGGCTCCC
7201 TTTAGGGTTC CGATTTAGTG CTTTACGGCA CCTCGACCCC AAAAAACTTG ATTTGGGTGA
7261 TGGTTCACGT AGTGGGCCAT CGCCCTGATA GACGGTTTTT CGCCCTTTGA CGTTGGAGTC
7321 CACGTTCTTT AATAGTGGAC TCTTGTTCCA AACTGGAACA ACACTCAACC CTATCTCGGG
7381 CTATTCTTTT GATTTATAAG GGATTTTGCC GATTTCGGAA CCACCATCAA ACAGGATTTT
7441 CGCCTGCTGG GGCAAACCAG CGTGGACCGC TTGCTGCAAC TCTCTCAGGG CCAGGCGGTG
7501 AAGGGCAATC AGCTGTTGCC CGTCTCACTG GTGAAAAGAA AAACCACCCT GGATCCAAGC
7561 TTGCAGGTGG CACTTTTCGG GGAAATGTGC GCGGAACCCC TATTTGTTTA TTTTTCTAAA
7621 TACATTCAAA TATGTATCCG CTCATGAGAC AATAACCCTG ATAAATGCTT CAATAATATT
7681 GAAAAAGGAA GAGTATGAGT ATTCAACATT TCCGTGTCGC CCTTATTCCC TTTTTTGCGG
7741 CATTTTGCCT TCCTGTTTTT GCTCACCCAG AAACGCTGGT GAAAGTAAAA GATGCTGAAG
7801 ATCAGTTGGG CGCACGAGTG GGTTACATCG AACTGGATCT CAACAGCGGT AAGATCCTTG
7861 AGAGTTTTCG CCCCGAAGAA CGTTTTCCAA TGATGAGCAC TTTTAAAGTT CTGCTATGTC
7921 ATACACTATT ATCCCGTATT GACGCCGGGC AAGAGCAACT CGGTCGCCGG GCGCGGTATT
7981 CTCAGAATGA CTTGGTTGAG TACTCACCAG TCACAGAAAA GCATCTTACG GATGGCATGA
8041 CAGTAAGAGA ATTATGCAGT GCTGCCATAA CCATGAGTGA TAACACTGCG GCCAACTTAC
8101 TTCTGACAAC GATCGGAGGA CCGAAGGAGC TAACCGCTTT TTTGCACAAC ATGGGGGATC
8161 ATGTAACTCG CCTTGATCGT TGGGAACCGG AGCTGAATGA AGCCATACCA AACGACGAGC
8221 GTGACACCAC GATGCCTGTA GCAATGCCAA CAACGTTGCG CAAACTATTA ACTGGCGAAC
8281 TACTTACTCT AGCTTCCCGG CAACAATTAA TAGACTGGAT GGAGGCGGAT AAAGTTGCAG
8341 GACCACTTCT GCGCTCGGCC CTTCCGGCTG GCTGGTTTAT TGCTGATAAA TCTGGAGCCG
8401 GTGAGCGTGG GTCTCGCGGT ATCATTGCAG CACTGGGGCC AGATGGTAAG CCCTCCCGTA
8461 TCGTAGTTAT CTACACGACG GGGAGTCAGG CAACTATGGA TGAACGAAAT AGACAGATCG
8521 CTGAGATAGG TGCCTCACTG ATTAAGCATT GGTAACTGTC AGACCAAGTT TACTCATATA
8581 TACTTTAGAT TGATTTAAAA CTTCATTTTT AATTTAAAAG GATCTAGGTG AAGATCCTTT
8641 TTGATAATCT CATGACCAAA ATCCCTTAAC GTGAGTTTTC GTTCCACTGT ACGTAAGACC
8701 CCCAAGCTTG TCGACTGAAT GGCGAATGGC GCTTTGCCTG GTTTCCGGCA CCAGAAGCGG
8761 TGCCGGAAAG CTGGCTGGAG TGCGATCTTC CTGAGGCCGA TACTGTCGTC GTCCCCTCAA
8821 ACTGGCAGAT GCACGGTTAC GATGCGCCCA TCTACACCAA CGTAACCTAT CCCATTACGG
8881 TCAATCCGCC GTTTGTTCCC ACGGAGAATC CGACGGGTTG TTACTCGCTC ACATTTAATG
8941 TTGATGAAAG CTGGCTACAG GAAGGCCAGA CGCGAATTAT TTTTGATGGC GTTCCTATTG
9001 GTTAAAAAAT GAGCTGATTT AACAAAAATT TAACGCGAAT TTTAACAAAA TATTAACGTT
9061 TACAATTTAA ATATTTGCTT ATACAATCTT CCTGTTTTTG GGGCTTTTCT GATTATCAAC
9121 CGGGGTACAT ATGATTGACA TGCTAGTTTT ACGATTACCG TTCATCGATT CTCTTGTTTG

9181 CTCCAGACTC TCAGGCAATG ACCTGATAGC CTTTGTAGAT CTCTCAAAAA TAGCTACCCT
9241 CTCCGGCATG AATTTATCAG CTAGAACGGT TGAATATCAT ATTGATGGTG ATTTGACTGT
9301 CTCCGGCCTT TCTCACCCTT TTGAATCTTT ACCTACACAT TACTCAGGCA TTGCATTTAA
9361 AATATATGAG GGTTCTAAAA ATTTTTATCC TTGCGTTGAA ATAAAGGCTT CTCCCGCAAA
9421 AGTATTACAG GGTCATAATG TTTTTGGTAC AACCGATTTA GCTTTATGCT CTGAGGCTTT
9481 ATTGCTTAAT TTTGCTAATT CTTTGCCTTG CCTGTATGAT TTATTGGATG TT

Table 200: Enzymes that either cut 15 or more human GLGs or have 5+-base recognition in FR3

Typical entry:

REname Recognition #sites

GLGid#:base# GLGid#:base# GLGid#:base#.....

BstEII Ggtnacc 2

1: 3 48: 3

There are 2 hits at base# 3

MaeIII gtnac 36

1: 4 2: 4 3: 4 4: 4 5: 4 6: 4

7: 4 8: 4 9: 4 10: 4 11: 4 37: 4

37: 58 38: 4 38: 58 39: 4 39: 58 40: 4

40: 58 41: 4 41: 58 42: 4 42: 58 43: 4

43: 58 44: 4 44: 58 45: 4 45: 58 46: 4

46: 58 47: 4 47: 58 48: 4 49: 4 50: 58

There are 24 hits at base# 4

Tsp45I gtsac 33

1: 4 2: 4 3: 4 4: 4 5: 4 6: 4

7: 4 8: 4 9: 4 10: 4 11: 4 37: 4

37: 58 38: 4 38: 58 39: 58 40: 4 40: 58

41: 58 42: 58 43: 4 43: 58 44: 4 44: 58

45: 4 45: 58 46: 4 46: 58 47: 4 47: 58

48: 4 49: 4 50: 58

There are 21 hits at base# 4

HphI tcacc 45

1: 5 2: 5 3: 5 4: 5 5: 5 6: 5

7: 5 8: 5 11: 5 12: 5 12: 11 13: 5

14: 5 15: 5 16: 5 17: 5 18: 5 19: 5

20: 5 21: 5 22: 5 23: 5 24: 5 25: 5

26: 5 27: 5 28: 5 29: 5 30: 5 31: 5

32: 5 33: 5 34: 5 35: 5 36: 5 37: 5

38: 5 40: 5 43: 5 44: 5 45: 5 46: 5

47: 5 48: 5 49: 5

There are 44 hits at base# 5

NlaIII CATG 26

1: 9 1: 42 2: 42 3: 9 3: 42 4: 9

4: 42 5: 9 5: 42 6: 42 6: 78 7: 9

7: 42 8: 21 8: 42 9: 42 10: 42 11: 42

12: 57 13: 48 13: 57 14: 57 31: 72 38: 9

48: 78 49: 78

There are 11 hits at base# 42

There are 1 hits at base# 48 Could cause raggedness.

BsaJI Ccnngg 37

1: 14 2: 14 5: 14 6: 14 7: 14 8: 14

8: 65 9: 14 10: 14 11: 14 12: 14 13: 14

14: 14 15: 65 17: 14 17: 65 18: 65 19: 65

20: 65 21: 65 22: 65 26: 65 29: 65 30: 65

33: 65 34: 65 35: 65 37: 65 38: 65 39: 65

40: 65 42: 65 43: 65 48: 65 49: 65 50: 65

51: 14

There are 23 hits at base# 65

There are 14 hits at base# 14

AluI AGct 42

1: 47 2: 47 3: 47 4: 47 5: 47 6: 47

7: 47 8: 47 9: 47 10: 47 11: 47 16: 63

23: 63 24: 63 25: 63 31: 63 32: 63 36: 63

37: 47 37: 52 38: 47 38: 52 39: 47 39: 52

40: 47 40: 52 41: 47 41: 52 42: 47 42: 52

43: 47 43: 52 44: 47 44: 52 45: 47 45: 52

46: 47 46: 52 47: 47 47: 52 49: 15 50: 47

There are 23 hits at base# 47

There are 11 hits at base# 52 Only 5 bases from 47

BlpI GCtnagc 21

1: 48 2: 48 3: 48 5: 48 6: 48 7: 48

8: 48 9: 48 10: 48 11: 48 37: 48 38: 48

39: 48 40: 48 41: 48 42: 48 43: 48 44: 48

45: 48 46: 48 47: 48

There are 21 hits at base# 48

MwoI GCNNNNNnngc 19

1: 48 2: 28 19: 36 22: 36 23: 36 24: 36
25: 36 26: 36 35: 36 37: 67 39: 67 40: 67
41: 67 42: 67 43: 67 44: 67 45: 67 46: 67
47: 67

There are 10 hits at base# 67
There are 7 hits at base# 36

DdeI Ctnag 71

1: 49 1: 58 2: 49 2: 58 3: 49 3: 58
3: 65 4: 49 4: 58 5: 49 5: 58 5: 65
6: 49 6: 58 6: 65 7: 49 7: 58 7: 65
8: 49 8: 58 9: 49 9: 58 9: 65 10: 49
10: 58 10: 65 11: 49 11: 58 11: 65 15: 58
16: 58 16: 65 17: 58 18: 58 20: 58 21: 58
22: 58 23: 58 23: 65 24: 58 24: 65 25: 58
25: 65 26: 58 27: 58 27: 65 28: 58 30: 58
31: 58 31: 65 32: 58 32: 65 35: 58 36: 58
36: 65 37: 49 38: 49 39: 26 39: 49 40: 49
41: 49 42: 26 42: 49 43: 49 44: 49 45: 49
46: 49 47: 49 48: 12 49: 12 51: 65

There are 29 hits at base# 58

There are 22 hits at base# 49 Only nine base from 58
There are 16 hits at base# 65 Only seven bases from 58

BglII Agatct 11

1: 61 2: 61 3: 61 4: 61 5: 61 6: 61
7: 61 9: 61 10: 61 11: 61 51: 47

There are 10 hits at base# 61

BstYI Rgatcy 12

1: 61 2: 61 3: 61 4: 61 5: 61 6: 61
7: 61 8: 61 9: 61 10: 61 11: 61 51: 47

There are 11 hits at base# 61

Hpy188I TCNga 17

1: 64 2: 64 3: 64 4: 64 5: 64 6: 64

7: 64 8: 64 9: 64 10: 64 11: 64 16: 57

20: 57 27: 57 35: 57 48: 67 49: 67

There are 11 hits at base# 64

There are 4 hits at base# 57

There are 2 hits at base# 67 Could be ragged.

MslI CAYNNnnRTG 44

1: 72 2: 72 3: 72 4: 72 5: 72 6: 72

7: 72 8: 72 9: 72 10: 72 11: 72 15: 72

17: 72 18: 72 19: 72 21: 72 23: 72 24: 72

25: 72 26: 72 28: 72 29: 72 30: 72 31: 72

32: 72 33: 72 34: 72 35: 72 36: 72 37: 72

38: 72 39: 72 40: 72 41: 72 42: 72 43: 72

44: 72 45: 72 46: 72 47: 72 48: 72 49: 72

50: 72 51: 72

There are 44 hits at base# 72

BsiEI CGRYcg 23

1: 74 3: 74 4: 74 5: 74 7: 74 8: 74

9: 74 10: 74 11: 74 17: 74 22: 74 30: 74

33: 74 34: 74 37: 74 38: 74 39: 74 40: 74

41: 74 42: 74 45: 74 46: 74 47: 74

There are 23 hits at base# 74

EaeI Yggccr 23

1: 74 3: 74 4: 74 5: 74 7: 74 8: 74

9: 74 10: 74 11: 74 17: 74 22: 74 30: 74

33: 74 34: 74 37: 74 38: 74 39: 74 40: 74

41: 74 42: 74 45: 74 46: 74 47: 74

There are 23 hits at base# 74

EagI Cggccg 23

1: 74 3: 74 4: 74 5: 74 7: 74 8: 74

9: 74 10: 74 11: 74 17: 74 22: 74 30: 74

33: 74 34: 74 37: 74 38: 74 39: 74 40: 74

41: 74 42: 74 45: 74 46: 74 47: 74

There are 23 hits at base# 74

HaeIII GGcc 27

1: 75 3: 75 4: 75 5: 75 7: 75 8: 75

9: 75 10: 75 11: 75 16: 75 17: 75 20: 75

22: 75 30: 75 33: 75 34: 75 37: 75 38: 75

39: 75 40: 75 41: 75 42: 75 45: 75 46: 75

47: 75 48: 63 49: 63

There are 25 hits at base# 75

Bst4CI ACNgt 65°C 63 Sites There is a third isoschismer

1: 86 2: 86 3: 86 4: 86 5: 86 6: 86

7: 34 7: 86 8: 86 9: 86 10: 86 11: 86

12: 86 13: 86 14: 86 15: 36 15: 86 16: 53

16: 86 17: 36 17: 86 18: 86 19: 86 20: 53

20: 86 21: 36 21: 86 22: 0 22: 86 23: 86

24: 86 25: 86 26: 86 27: 53 27: 86 28: 36

28: 86 29: 86 30: 86 31: 86 32: 86 33: 36

33: 86 34: 86 35: 53 35: 86 36: 86 37: 86

38: 86 39: 86 40: 86 41: 86 42: 86 43: 86

44: 86 45: 86 46: 86 47: 86 48: 86 49: 86

50: 86 51: 0 51: 86

There are 51 hits at base# 86 All the other sites are well away

HpyCH4III ACNgt 63

1: 86 2: 86 3: 86 4: 86 5: 86 6: 86

7: 34 7: 86 8: 86 9: 86 10: 86 11: 86

12: 86 13: 86 14: 86 15: 36 15: 86 16: 53

16: 86 17: 36 17: 86 18: 86 19: 86 20: 53

20: 86 21: 36 21: 86 22: 0 22: 86 23: 86

24: 86 25: 86 26: 86 27: 53 27: 86 28: 36

28: 86 29: 86 30: 86 31: 86 32: 86 33: 36

33: 86 34: 86 35: 53 35: 86 36: 86 37: 86

38: 86 39: 86 40: 86 41: 86 42: 86 43: 86

44: 86 45: 86 46: 86 47: 86 48: 86 49: 86

50: 86 51: 0 51: 86

There are 51 hits at base# 86

HinfI Gantc 43

2: 2 3: 2 4: 2 5: 2 6: 2 7: 2

8: 2 9: 2 9: 22 10: 2 11: 2 15: 2

16: 2 17: 2 18: 2 19: 2 19: 22 20: 2

21: 2 23: 2 24: 2 25: 2 26: 2 27: 2

28: 2 29: 2 30: 2 31: 2 32: 2 33: 2

33: 22 34: 22 35: 2 36: 2 37: 2 38: 2

40: 2 43: 2 44: 2 45: 2 46: 2 47: 2

50: 60

There are 38 hits at base# 2

MlyI GAGTCNNNNNn 18

2: 2 3: 2 4: 2 5: 2 6: 2 7: 2

8: 2 9: 2 10: 2 11: 2 37: 2 38: 2

40: 2 43: 2 44: 2 45: 2 46: 2 47: 2

There are 18 hits at base# 2

PleI gagtc 18

2: 2 3: 2 4: 2 5: 2 6: 2 7: 2

8: 2 9: 2 10: 2 11: 2 37: 2 38: 2

40: 2 43: 2 44: 2 45: 2 46: 2 47: 2

There are 18 hits at base# 2

AciI Ccgc 24

2: 26 9: 14 10: 14 11: 14 27: 74 37: 62

37: 65 38: 62 39: 65 40: 62 40: 65 41: 65

42: 65 43: 62 43: 65 44: 62 44: 65 45: 62

46: 62 47: 62 47: 65 48: 35 48: 74 49: 74

There are 8 hits at base# 62

There are 8 hits at base# 65

There are 3 hits at base# 14

There are 3 hits at base# 74

There are 1 hits at base# 26

There are 1 hits at base# 35

-"- Gcgg 11

8: 91 9: 16 10: 16 11: 16 37: 67 39: 67

40: 67 42: 67 43: 67 45: 67 46: 67

There are 7 hits at base# 67

There are 3 hits at base# 16

There are 1 hits at base# 91

BsiHKAI GWGCWc 20

2: 30 4: 30 6: 30 7: 30 9: 30 10: 30

12: 89 13: 89 14: 89 37: 51 38: 51 39: 51

40: 51 41: 51 42: 51 43: 51 44: 51 45: 51

46: 51 47: 51

There are 11 hits at base# 51

Bsp1286I GDGCHc 20

2: 30 4: 30 6: 30 7: 30 9: 30 10: 30

12: 89 13: 89 14: 89 37: 51 38: 51 39: 51

40: 51 41: 51 42: 51 43: 51 44: 51 45: 51

46: 51 47: 51

There are 11 hits at base# 51

HgiAI GWGCWc 20

2: 30 4: 30 6: 30 7: 30 9: 30 10: 30

12: 89 13: 89 14: 89 37: 51 38: 51 39: 51

40: 51 41: 51 42: 51 43: 51 44: 51 45: 51

46: 51 47: 51

There are 11 hits at base# 51

BsoFI GCngc 26

2: 53 3: 53 5: 53 6: 53 7: 53 8: 53

8: 91 9: 53 10: 53 11: 53 31: 53 36: 36

37: 64 39: 64 40: 64 41: 64 42: 64 43: 64

44: 64 45: 64 46: 64 47: 64 48: 53 49: 53

50: 45 51: 53

There are 13 hits at base# 53

There are 10 hits at base# 64

TseI Gcwgc 17

2: 53 3: 53 5: 53 6: 53 7: 53 8: 53

9: 53 10: 53 11: 53 31: 53 36: 36 45: 64
46: 64 48: 53 49: 53 50: 45 51: 53
There are 13 hits at base# 53

MnlI gagg 34
3: 67 3: 95 4: 51 5: 16 5: 67 6: 67
7: 67 8: 67 9: 67 10: 67 11: 67 15: 67
16: 67 17: 67 19: 67 20: 67 21: 67 22: 67
23: 67 24: 67 25: 67 26: 67 27: 67 28: 67
29: 67 30: 67 31: 67 32: 67 33: 67 34: 67
35: 67 36: 67 50: 67 51: 67
There are 31 hits at base# 67

HpyCH4V TGca 34
5: 90 6: 90 11: 90 12: 90 13: 90 14: 90
15: 44 16: 44 16: 90 17: 44 18: 90 19: 44
20: 44 21: 44 22: 44 23: 44 24: 44 25: 44
26: 44 27: 44 27: 90 28: 44 29: 44 33: 44
34: 44 35: 44 35: 90 36: 38 48: 44 49: 44
50: 44 50: 90 51: 44 51: 52
There are 21 hits at base# 44
There are 1 hits at base# 52

AccI GTmkac 13 5-base recognition
7: 37 11: 24 37: 16 38: 16 39: 16 40: 16
41: 16 42: 16 43: 16 44: 16 45: 16 46: 16
47: 16
There are 11 hits at base# 16

SacII CCGCgg 8 6-base recognition
9: 14 10: 14 11: 14 37: 65 39: 65 40: 65
42: 65 43: 65
There are 5 hits at base# 65
There are 3 hits at base# 14

TfiI Gawtc 24
9: 22 15: 2 16: 2 17: 2 18: 2 19: 2
19: 22 20: 2 21: 2 23: 2 24: 2 25: 2

26: 2 27: 2 28: 2 29: 2 30: 2 31: 2
32: 2 33: 2 33: 22 34: 22 35: 2 36: 2
There are 20 hits at base# 2

BsmAI Nnnnnngagac 19
15: 11 16: 11 20: 11 21: 11 22: 11 23: 11
24: 11 25: 11 26: 11 27: 11 28: 11 28: 56
30: 11 31: 11 32: 11 35: 11 36: 11 44: 87
48: 87
There are 16 hits at base# 11

BpmI ctccag 19
15: 12 16: 12 17: 12 18: 12 20: 12 21: 12
22: 12 23: 12 24: 12 25: 12 26: 12 27: 12
28: 12 30: 12 31: 12 32: 12 34: 12 35: 12
36: 12
There are 19 hits at base# 12

XmnI GAANNnnttc 12
37: 30 38: 30 39: 30 40: 30 41: 30 42: 30
43: 30 44: 30 45: 30 46: 30 47: 30 50: 30
There are 12 hits at base# 30

BsrI NCcagt 12
37: 32 38: 32 39: 32 40: 32 41: 32 42: 32
43: 32 44: 32 45: 32 46: 32 47: 32 50: 32
There are 12 hits at base# 32

BanII GRGCYc 11
37: 51 38: 51 39: 51 40: 51 41: 51 42: 51
43: 51 44: 51 45: 51 46: 51 47: 51
There are 11 hits at base# 51

Ecl136I GAGctc 11
37: 51 38: 51 39: 51 40: 51 41: 51 42: 51
43: 51 44: 51 45: 51 46: 51 47: 51
There are 11 hits at base# 51

SacI GAGCTc 11

```
37: 51   38: 51   39: 51   40: 51   41: 51   42: 51
43: 51   44: 51   45: 51   46: 51   47: 51
There are  11 hits at base# 51
```

Table 206: Synthetic 3-23 FR3 of human heavy chains showning positions of possible cleavage sites

```
! Sites engineered into the synthetic gene are shown in upper case DNA
! with the RE name between vertical bars (as in | XbaI  |).
! RERSs frequently found in GLGs are shown below the synthetic sequence
! with the name to the right (as in gtn ac=MaeIII(24), indicating that
! 24 of the 51 GLGs contain the site).
!
!                                                                  |---FR3---
!                                                                   89  90 (codon # in
!                                                                    R   F  synthetic 3-23)
!  Allowed DNA                                                     |cgc|ttc|  6
!                                                                  |cgn|tty|
!                                                                  |agr|
!                                                                    ga ntc = HinfI(38)
!                                                                    ga gtc = PleI(18)
!                                                                    ga wtc = TfiI(20)
!                                                                       gtn ac = MaeIII(24)
!                                                                       gts ac = Tsp45I(21)
!                                                                        tc acc = HphI(44)
!
!       --------FR3-------------------------------------------------
!         91  92  93  94  95  96  97  98  99 100 101 102 103 104 105
!          T   I   S   R   D   N   S   K   N   T   L   Y   L   Q   M
!       |act|atc|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|  51
!allowed|acn|ath|tcn|cgn|gay|aay|tcn|aar|aay|acn|ttr|tay|ttr|car|atg|
!               |agy|agr|       |agy|           |ctn|   |ctn|
!               |     ga|gac = BsmAI(16)                          ag ct = AluI(23)
!              c|tcc ag = BpmI(19)                                g ctn agc = BlpI(21)
!               |       |             g aan nnn ttc = XmnI(12)
!               | XbaI  |                                  tg ca = HpyCH4V(21)
!
!       ---FR3-----------------------------------------------------------|
!        106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
!         N   S   L   R   A   E   D   T   A   V   Y   Y   C   A   K
!       |aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|tgc|gct|aaa|  96
!allowed|aay|tcn|ttr|cgn|gcn|gar|gay|acn|gcn|gtn|tay|tay|tgy|gcn|aar|
!           |agy|ctn|agr|             |     |
!             |     |   cc nng g = BsaJI(23)        ac ngt = Bst4CI(51)
!             |    aga tct = BglII(10)     |        ac ngt = HpyCH4III(51)
!             |    Rga tcY = BstYI(11)     |        ac ngt = TaaI(51)
!             |     |            c ayn nnn rtc = MslI(44)
!             |     |               cg ryc g = BsiEI(23)
!             |     |               yg gcc r = EaeI(23)
!             |     |               cg gcc g = EagI(23)
!             |     |               |g gcc = HaeIII(25)
!             |     |     gag g = MnlI(31)|
!             |AflII |               | PstI |
```

Table 217: Human HC GLG FR1 Sequences

VH Exon - Nucleotide sequence alignment

VH1

1-02 CAG GTG CAG CTG GTG CAG TCT GGG GCT GAG GTG AAG AAG CCT GGG GCC TCA GTG AAG
GTC TCC TGC AAG GCT TCT GGA TAC ACC TTC ACC

1-03 cag gtC cag ctT gtg cag tct ggg gct gag gtg aag aag cct ggg gcc tca gtg aag
gtT tcc tgc aag gct tct gga tac acc ttc acT

1-08 cag gtg cag ctg gtg cag tct ggg gct gag gtg aag aag cct ggg gcc tca gtg aag
gtc tcc tgc aag gct tct gga tac acc ttc acc

1-18 cag gtT cag ctg gtg cag tct ggA gct gag gtg aag aag cct ggg gcc tca gtg aag
gtc tcc tgc aag gct tct ggT tac acc ttT acc

1-24 cag gtC cag ctg gtA cag tct ggg gct gag gtg aag aag cct ggg gcc tca gtg aag
gtc tcc tgc aag gTt tcC gga tac acc Ctc acT

1-45 cag Atg cag ctg gtg cag tct ggg gct gag gtg aag aag Act ggg Tcc tca gtg aag
gtT tcc tgc aag gct tcC gga tac acc ttc acc

1-46 cag gtg cag ctg gtg cag tct ggg gct gag gtg aag aag cct ggg gcc tca gtg aag
gtT tcc tgc aag gcA tct gga tac acc ttc acc

1-58 caA Atg cag ctg gtg cag tct ggg Cct gag gtg aag aag cct ggg Acc tca gtg aag
gtc tcc tgc aag gct tct gga tTc acc ttT acT

1-69 cag gtg cag ctg gtg cag tct ggg gct gag gtg aag aag cct ggg Tcc tcG gtg aag
gtc tcc tgc aag gct tct gga GGc acc ttc aGc

1-e cag gtg cag ctg gtg cag tct ggg gct gag gtg aag aag cct ggg Tcc tcG gtg aag
gtc tcc tgc aag gct tct gga GGc acc ttc aGc

1-f Gag gtC cag ctg gtA cag tct ggg gct gag gtg aag aag cct ggg gcT Aca gtg aaA
Atc tcc tgc aag gTt tct gga tac acc ttc acc

VH2

2-05 CAG ATC ACC TTG AAG GAG TCT GGT CCT ACG CTG GTG AAA CCC ACA CAG ACC CTC ACG
CTG ACC TGC ACC TTC TCT GGG TTC TCA CTC AGC

2-26 cag Gtc acc ttg aag gag tct ggt cct GTg ctg gtg aaa ccc aca Gag acc ctc acg
ctg acc tgc acc Gtc tct ggg ttc tca ctc agc

2-70 cag Gtc acc ttg aag gag tct ggt cct Gcg ctg gtg aaa ccc aca cag acc ctc acA
ctg acc tgc acc ttc tct ggg ttc tca ctc agc

VH3

3-07 GAG GTG CAG CTG GTG GAG TCT GGG GGA GGC TTG GTC CAG CCT GGG GGG TCC CTG AGA
CTC TCC TGT GCA GCC TCT GGA TTC ACC TTT AGT

3-09 gaA gtg cag ctg gtg gag tct ggg gga ggc ttg gtA cag cct ggC Agg tcc ctg aga
ctc tcc tgt gca gcc tct gga ttc acc ttt GAt

3-11 Cag gtg cag ctg gtg gag tct ggg gga ggc ttg gtc Aag cct ggA ggg tcc ctg aga
ctc tcc tgt gca gcc tct gga ttc acc ttC agt

3-13 gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtA cag cct ggg ggg tcc ctg aga
ctc tcc tgt gca gcc tct gga ttc acc ttC agt

3-15 gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtA Aag cct ggg ggg tcc ctT aga
ctc tcc tgt gca gcc tct gga ttc acT ttC agt

3-20 gag gtg cag ctg gtg gag tct ggg gga ggT Gtg gtA cGg cct ggg ggg tcc ctg aga

ctc tcc tgt gca gcc tct gga ttc acc ttt GAt

3-21 gag gtg cag ctg gtg gag tct ggg gga ggc Ctg gtc Aag cct ggg ggg tcc ctg aga
ctc tcc tgt gca gcc tct gga ttc acc ttC agt

3-23 gag gtg cag ctg Ttg gag tct ggg gga ggc ttg gtA cag cct ggg ggg tcc ctg aga
ctc tcc tgt gca gcc tct gga ttc acc ttt agC

3-30 Cag gtg cag ctg gtg gag tct ggg gga ggc Gtg gtc cag cct ggg Agg tcc ctg aga
ctc tcc tgt gca gcc tct gga ttc acc ttC agt

3-30.3 Cag gtg cag ctg gtg gag tct ggg gga ggc Gtg gtc cag cct ggg Agg tcc ctg aga
ctc tcc tgt gca gcc tct gga ttc acc ttC agt

3-30.5 Cag gtg cag ctg gtg gag tct ggg gga ggc Gtg gtc cag cct ggg Agg tcc ctg aga
ctc tcc tgt gca gcc tct gga ttc acc ttC agt

3-33 Cag gtg cag ctg gtg gag tct ggg gga ggc Gtg gtc cag cct ggg Agg tcc ctg aga
ctc tcc tgt gca gcG tct gga ttc acc ttC agt

3-43 gaA gtg cag ctg gtg gag tct ggg gga gTc Gtg gtA cag cct ggg ggg tcc ctg aga
ctc tcc tgt gca gcc tct gga ttc acc ttt GAt

3-48 gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtA cag cct ggg ggg tcc ctg aga
ctc tcc tgt gca gcc tct gga ttc acc ttC agt

3-49 gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtA cag ccA ggg Cgg tcc ctg aga
ctc tcc tgt Aca gcT tct gga ttc acc ttt Ggt

3-53 gag gtg cag ctg gtg gag Act ggA gga ggc ttg Atc cag cct ggg ggg tcc ctg aga
ctc tcc tgt gca gcc tct ggG ttc acc GtC agt

3-64 gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtc cag cct ggg ggg tcc ctg aga
ctc tcc tgt gca gcc tct gga ttc acc ttC agt

3-66 gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtc cag cct ggg ggg tcc ctg aga
ctc tcc tgt gca gcc tct gga ttc acc GtC agt

3-72 gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtc cag cct ggA ggg tcc ctg aga
ctc tcc tgt gca gcc tct gga ttc acc ttC agt

3-73 gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtc cag cct ggg ggg tcc ctg aAa
ctc tcc tgt gca gcc tct ggG ttc acc ttC agt

3-74 gag gtg cag ctg gtg gag tcC ggg gga ggc ttA gtT cag cct ggg ggg tcc ctg aga
ctc tcc tgt gca gcc tct gga ttc acc ttC agt

3-d gag gtg cag ctg gtg gag tct Cgg gga gTc ttg gtA cag cct ggg ggg tcc ctg aga
ctc tcc tgt gca gcc tct gga ttc acc GtC agt

VH4

4-04 CAG GTG CAG CTG CAG GAG TCG GGC CCA GGA CTG GTG AAG CCT TCG GGG ACC CTG TCC
CTC ACC TGC GCT GTC TCT GGT GGC TCC ATC AGC

4-28 cag gtg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcg gAC acc ctg tcc
ctc acc tgc gct gtc tct ggt TAc tcc atc agc

4-30.1 cag gtg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcA CAg acc ctg tcc
ctc acc tgc Act gtc tct ggt ggc tcc atc agc

4-30.2 cag Ctg cag ctg cag gag tcC ggc Tca gga ctg gtg aag cct tcA CAg acc ctg tcc
ctc acc tgc gct gtc tct ggt ggc tcc atc agc

4-30.4 cag gtg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcA CAg acc ctg tcc
ctc acc tgc Act gtc tct ggt ggc tcc atc agc

4-31 cag gtg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcA CAg acc ctg tcc
ctc acc tgc Act gtc tct ggt ggc tcc atc agc

4-34 cag gtg cag ctA cag Cag tGg ggc Gca gga ctg Ttg aag cct tcg gAg acc ctg tcc
ctc acc tgc gct gtc tAt ggt ggG tcc Ttc agT

4-39 cag Ctg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcg gAg acc ctg tcc
ctc acc tgc Act gtc tct ggt ggc tcc atc agc

4-59 cag gtg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcg gAg acc ctg tcc
ctc acc tgc Act gtc tct ggt ggc tcc atc agT

4-61 cag gtg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcg gAg acc ctg tcc
ctc acc tgc Act gtc tct ggt ggc tcc Gtc agc

4-b cag gtg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcg gAg acc ctg tcc
ctc acc tgc gct gtc tct ggt TAc tcc atc agc

VH5

5-51 GAG GTG CAG CTG GTG CAG TCT GGA GCA GAG GTG AAA AAG CCC GGG GAG TCT CTG AAG
ATC TCC TGT AAG GGT TCT GGA TAC AGC TTT ACC

5-a gaA gtg cag ctg gtg cag tct gga gca gag gtg aaa aag ccc ggg gag tct ctg aGg
atc tcc tgt aag ggt tct gga tac agc ttt acc

VH6

6-1 CAG GTA CAG CTG CAG CAG TCA GGT CCA GGA CTG GTG AAG CCC TCG CAG ACC CTC TCA
CTC ACC TGT GCC ATC TCC GGG GAC AGT GTC TCT

VH7

7-4.1 CAG GTG CAG CTG GTG CAA TCT GGG TCT GAG TTG AAG AAG CCT GGG GCC TCA GTG AAG
GTT TCC TGC AAG GCT TCT GGA TAC ACC TTC ACT

Table 220: RERS sites in Human HC GLG FR1s where there are at least 20 GLGs cut

BsgI GTGCAG 71 (cuts 16/14 bases to right)

1: 4 1: 13 2: 13 3: 4 3: 13 4: 13
6: 13 7: 4 7: 13 8: 13 9: 4 9: 13
10: 4 10: 13 15: 4 15: 65 16: 4 16: 65
17: 4 17: 65 18: 4 18: 65 19: 4 19: 65
20: 4 20: 65 21: 4 21: 65 22: 4 22: 65
23: 4 23: 65 24: 4 24: 65 25: 4 25: 65
26: 4 26: 65 27: 4 27: 65 28: 4 28: 65
29: 4 30: 4 30: 65 31: 4 31: 65 32: 4
32: 65 33: 4 33: 65 34: 4 34: 65 35: 4
35: 65 36: 4 36: 65 37: 4 38: 4 39: 4
41: 4 42: 4 43: 4 45: 4 46: 4 47: 4
48: 4 48: 13 49: 4 49: 13 51: 4

There are 39 hits at base# 4

There are 21 hits at base# 65

-"- ctgcac 9

12: 63 13: 63 14: 63 39: 63 41: 63 42: 63
44: 63 45: 63 46: 63

BbvI GCAGC 65

1: 6 3: 6 6: 6 7: 6 8: 6 9: 6
10: 6 15: 6 15: 67 16: 6 16: 67 17: 6
17: 67 18: 6 18: 67 19: 6 19: 67 20: 6
20: 67 21: 6 21: 67 22: 6 22: 67 23: 6
23: 67 24: 6 24: 67 25: 6 25: 67 26: 6
26: 67 27: 6 27: 67 28: 6 28: 67 29: 6
30: 6 30: 67 31: 6 31: 67 32: 6 32: 67
33: 6 33: 67 34: 6 34: 67 35: 6 35: 67
36: 6 36: 67 **37:** 6 **38:** 6 **39:** 6 **40:** 6
41: 6 **42:** 6 43: 6 **44:** 6 **45:** 6 **46:** 6
47: 6 48: 6 49: 6 **50:** 12 51: 6

There are 43 hits at base# 6 Bolded sites very near sites listed below

There are 21 hits at base# 67

-"- gctgc 13

37: 9 **38:** 9 **39:** 9 **40:** 3 **40:** 9 **41:** 9
42: 9 **44:** 3 **44:** 9 **45:** 9 **46:** 9 **47:** 9

50: 9

There are 11 hits at base# 9

BsoFI GCngc 78

1: 6	3: 6	6: 6	7: 6	8: 6	9: 6
10: 6	15: 6	15: 67	16: 6	16: 67	17: 6
17: 67	18: 6	18: 67	19: 6	19: 67	20: 6
20: 67	21: 6	21: 67	22: 6	22: 67	23: 6
23: 67	24: 6	24: 67	25: 6	25: 67	26: 6
26: 67	27: 6	27: 67	28: 6	28: 67	29: 6
30: 6	30: 67	31: 6	31: 67	32: 6	32: 67
33: 6	33: 67	34: 6	34: 67	35: 6	35: 67
36: 6	36: 67	37: 6	37: 9	38: 6	38: 9
39: 6	39: 9	40: 3	40: 6	40: 9	41: 6
41: 9	42: 6	42: 9	43: 6	44: 3	44: 6
44: 9	45: 6	45: 9	46: 6	46: 9	47: 6
47: 9	48: 6	49: 6	50: 9	50: 12	51: 6

There are 43 hits at base# 6 These often occur together.

There are 11 hits at base# 9

There are 2 hits at base# 3

There are 21 hits at base# 67

TseI Gcwgc 78

1: 6	3: 6	6: 6	7: 6	8: 6	9: 6
10: 6	15: 6	15: 67	16: 6	16: 67	17: 6
17: 67	18: 6	18: 67	19: 6	19: 67	20: 6
20: 67	21: 6	21: 67	22: 6	22: 67	23: 6
23: 67	24: 6	24: 67	25: 6	25: 67	26: 6
26: 67	27: 6	27: 67	28: 6	28: 67	29: 6
30: 6	30: 67	31: 6	31: 67	32: 6	32: 67
33: 6	33: 67	34: 6	34: 67	35: 6	35: 67
36: 6	36: 67	37: 6	37: 9	38: 6	38: 9
39: 6	39: 9	40: 3	40: 6	40: 9	41: 6
41: 9	42: 6	42: 9	43: 6	44: 3	44: 6
44: 9	45: 6	45: 9	46: 6	46: 9	47: 6
47: 9	48: 6	49: 6	50: 9	50: 12	51: 6

There are 43 hits at base# 6 Often together.

There are 11 hits at base# 9

There are 2 hits at base# 3

There are 1 hits at base# 12

There are 21 hits at base# 67

MspAlI CMGckg 48

1: 7	3: 7	4: 7	5: 7	6: 7	7: 7
8: 7	9: 7	10: 7	11: 7	15: 7	16: 7
17: 7	18: 7	19: 7	20: 7	21: 7	22: 7
23: 7	24: 7	25: 7	26: 7	27: 7	28: 7
29: 7	30: 7	31: 7	32: 7	33: 7	34: 7
35: 7	36: 7	37: 7	38: 7	39: 7	40: 1
40: 7	41: 7	42: 7	44: 1	44: 7	45: 7
46: 7	47: 7	48: 7	49: 7	50: 7	51: 7

There are 46 hits at base# 7

PvuII CAGctg 48

1: 7	**3: 7**	**4: 7**	**5: 7**	**6: 7**	**7: 7**
8: 7	**9: 7**	**10: 7**	**11: 7**	**15: 7**	**16: 7**
17: 7	**18: 7**	**19: 7**	**20: 7**	**21: 7**	**22: 7**
23: 7	**24: 7**	**25: 7**	**26: 7**	**27: 7**	**28: 7**
29: 7	**30: 7**	**31: 7**	**32: 7**	**33: 7**	**34: 7**
35: 7	**36: 7**	**37: 7**	**38: 7**	**39: 7**	**40: 1**
40: 7	**41: 7**	**42: 7**	**44: 1**	**44: 7**	**45: 7**
46: 7	**47: 7**	**48: 7**	**49: 7**	**50: 7**	**51: 7**

There are 46 hits at base# 7

There are 2 hits at base# 1

AluI AGct 54

1: 8	2: 8	3: 8	4: 8	4: 24	5: 8
6: 8	7: 8	8: 8	9: 8	10: 8	11: 8
15: 8	16: 8	17: 8	18: 8	19: 8	20: 8
21: 8	22: 8	23: 8	24: 8	25: 8	26: 8
27: 8	28: 8	29: 8	29: 69	30: 8	31: 8
32: 8	33: 8	34: 8	35: 8	36: 8	37: 8
38: 8	39: 8	40: 2	40: 8	41: 8	42: 8
43: 8	44: 2	44: 8	45: 8	46: 8	47: 8
48: 8	48: 82	49: 8	49: 82	50: 8	51: 8

There are 48 hits at base# 8

There are 2 hits at base# 2

DdeI Ctnag 48

1: 26 1: 48 2: 26 2: 48 3: 26 3: 48
4: 26 4: 48 5: 26 5: 48 6: 26 6: 48
7: 26 7: 48 8: 26 8: 48 9: 26 10: 26
11: 26 12: 85 13: 85 14: 85 15: 52 16: 52
17: 52 18: 52 19: 52 20: 52 21: 52 22: 52
23: 52 24: 52 25: 52 26: 52 27: 52 28: 52
29: 52 30: 52 31: 52 32: 52 33: 52 35: 30
35: 52 36: 52 40: 24 49: 52 51: 26 51: 48

There are 22 hits at base# 52 52 and 48 never together.

There are 9 hits at base# 48

There are 12 hits at base# 26 26 and 24 never together.

HphI tcacc 42

1: 86 3: 86 6: 86 7: 86 8: 80 11: 86
12: 5 13: 5 14: 5 15: 80 16: 80 17: 80
18: 80 20: 80 21: 80 22: 80 23: 80 24: 80
25: 80 26: 80 27: 80 28: 80 29: 80 30: 80
31: 80 32: 80 33: 80 34: 80 35: 80 36: 80
37: 59 38: 59 39: 59 40: 59 41: 59 42: 59
43: 59 44: 59 45: 59 46: 59 47: 59 50: 59

There are 22 hits at base# 80 80 and 86 never together

There are 5 hits at base# 86

There are 12 hits at base# 59

BssKI Nccngg 50

1: 39 2: 39 3: 39 4: 39 5: 39 7: 39
8: 39 9: 39 10: 39 11: 39 15: 39 16: 39
17: 39 18: 39 19: 39 20: 39 21: 29 21: 39
22: 39 23: 39 24: 39 25: 39 26: 39 27: 39
28: 39 29: 39 30: 39 31: 39 32: 39 33: 39
34: 39 35: 19 35: 39 36: 39 37: 24 38: 24
39: 24 41: 24 42: 24 44: 24 45: 24 46: 24
47: 24 48: 39 48: 40 49: 39 49: 40 50: 24
50: 73 51: 39

There are 35 hits at base# 39 39 and 40 together twice.

There are 2 hits at base# 40

BsaJI Ccnngg 47

1: 40 2: 40 3: 40 4: 40 5: 40 7: 40
8: 40 9: 40 9: 47 10: 40 10: 47 11: 40
15: 40 18: 40 19: 40 20: 40 21: 40 22: 40
23: 40 24: 40 25: 40 26: 40 27: 40 28: 40
29: 40 30: 40 31: 40 32: 40 34: 40 35: 20
35: 40 36: 40 37: 24 38: 24 39: 24 41: 24
42: 24 44: 24 45: 24 46: 24 47: 24 48: 40
48: 41 49: 40 49: 41 50: 74 51: 40

There are 32 hits at base# 40 40 and 41 together twice

There are 2 hits at base# 41

There are 9 hits at base# 24

There are 2 hits at base# 47

BstNI CCwgg 44

PspGI ccwgg

ScrFI($M.HpaII) CCwgg

1: 40 2: 40 3: 40 4: 40 5: 40 7: 40
8: 40 9: 40 10: 40 11: 40 15: 40 16: 40
17: 40 18: 40 19: 40 20: 40 21: 30 21: 40
22: 40 23: 40 24: 40 25: 40 26: 40 27: 40
28: 40 29: 40 30: 40 31: 40 32: 40 33: 40
34: 40 35: 40 36: 40 37: 25 38: 25 39: 25
41: 25 42: 25 44: 25 45: 25 46: 25 47: 25
50: 25 51: 40

There are 33 hits at base# 40

ScrFI CCngg 50

1: 40 2: 40 3: 40 4: 40 5: 40 7: 40
8: 40 9: 40 10: 40 11: 40 15: 40 16: 40
17: 40 18: 40 19: 40 20: 40 21: 30 21: 40
22: 40 23: 40 24: 40 25: 40 26: 40 27: 40
28: 40 29: 40 30: 40 31: 40 32: 40 33: 40
34: 40 35: 20 35: 40 36: 40 37: 25 38: 25
39: 25 41: 25 42: 25 44: 25 45: 25 46: 25
47: 25 48: 40 48: 41 49: 40 49: 41 50: 25
50: 74 51: 40

There are 35 hits at base# 40

There are 2 hits at base# 41

EcoO109I RGgnccy 34

1: 43 2: 43 3: 43 4: 43 5: 43 6: 43
7: 43 8: 43 9: 43 10: 43 15: 46 16: 46
17: 46 18: 46 19: 46 20: 46 21: 46 22: 46
23: 46 24: 46 25: 46 26: 46 27: 46 28: 46
30: 46 31: 46 32: 46 33: 46 34: 46 35: 46
36: 46 37: 46 43: 79 51: 43

There are 22 hits at base# 46 46 and 43 never together

There are 11 hits at base# 43

NlaIV GGNncc 71

1: 43 2: 43 3: 43 4: 43 5: 43 6: 43
7: 43 8: 43 9: 43 9: 79 10: 43 10: 79
15: 46 15: 47 16: 47 17: 46 17: 47 18: 46
18: 47 19: 46 19: 47 20: 46 20: 47 21: 46
21: 47 22: 46 22: 47 23: 47 24: 47 25: 47
26: 47 27: 46 27: 47 28: 46 28: 47 29: 47
30: 46 30: 47 31: 46 31: 47 32: 46 32: 47
33: 46 33: 47 34: 46 34: 47 35: 46 35: 47
36: 46 36: 47 37: 21 37: 46 37: 47 37: 79
38: 21 39: 21 39: 79 40: 79 41: 21 41: 79
42: 21 42: 79 43: 79 44: 21 44: 79 45: 21
45: 79 46: 21 46: 79 47: 21 51: 43

There are 23 hits at base# 47 46 & 47 often together

There are 17 hits at base# 46 There are 11 hits at base# 43

Sau96I Ggncc 70

1: 44 2: 3 2: 44 3: 44 4: 44 5: 3 5: 44 6: 44
7: 44 8: 22 8: 44 9: 44 10: 44 11: 3 12: 22 13: 22
14: 22 15: 33 15: 47 16: 47 17: 47 18: 47 19: 47 20: 47
21: 47 22: 47 23: 33 23: 47 24: 33 24: 47 25: 33 25: 47
26: 33 26: 47 27: 47 28: 47 29: 47 30: 47 31: 33 31: 47
32: 33 32: 47 33: 33 33: 47 34: 33 34: 47 35: 47 36: 47
37: 21 37: 22 37: 47 38: 21 38: 22 39: 21 39: 22 41: 21
41: 22 42: 21 42: 22 43: 80 44: 21 44: 22 45: 21 45: 22
46: 21 46: 22 47: 21 47: 22 50: 22 51: 44

There are 23 hits at base# 47 These do not occur together.

There are 11 hits at base# 44

There are 14 hits at base# 22 These do occur together.
There are 9 hits at base# 21

BsmAI GTCTCNnnnn 22

1: 58 3: 58 4: 58 5: 58 8: 58 9: 58
10: 58 13: 70 36: 18 37: 70 38: 70 39: 70
40: 70 41: 70 42: 70 44: 70 45: 70 46: 70
47: 70 48: 48 49: 48 50: 85
There are 11 hits at base# 70

-"- Nnnnnngagac 27

13: 40 15: 48 16: 48 17: 48 18: 48 20: 48
21: 48 22: 48 23: 48 24: 48 25: 48 26: 48
27: 48 28: 48 29: 48 30: 10 30: 48 31: 48
32: 48 33: 48 35: 48 36: 48 43: 40 44: 40
45: 40 46: 40 47: 40
There are 20 hits at base# 48

AvaII Ggwcc 44
Sau96I($M.HaeIII) Ggwcc 44

2: 3 5: 3 6: 44 8: 44 9: 44 10: 44
11: 3 12: 22 13: 22 14: 22 15: 33 15: 47
16: 47 17: 47 18: 47 19: 47 20: 47 21: 47
22: 47 23: 33 23: 47 24: 33 24: 47 25: 33
25: 47 26: 33 26: 47 27: 47 28: 47 29: 47
30: 47 31: 33 31: 47 32: 33 32: 47 33: 33
33: 47 34: 33 34: 47 35: 47 36: 47 37: 47
43: 80 50: 22
There are 23 hits at base# 47 44 & 47 never together
There are 4 hits at base# 44

PpuMI RGgwccy 27

6: 43 8: 43 9: 43 10: 43 15: 46 16: 46
17: 46 18: 46 19: 46 20: 46 21: 46 22: 46
23: 46 24: 46 25: 46 26: 46 27: 46 28: 46
30: 46 31: 46 32: 46 33: 46 34: 46 35: 46
36: 46 37: 46 43: 79
There are 22 hits at base# 46 43 and 46 never occur together.
There are 4 hits at base# 43

BsmFI GGGAC 3

8: 43 37: 46 50: 77

-"- gtccc 33

15: 48 16: 48 17: 48 1: 0 1: 0 20: 48

21: 48 22: 48 23: 48 24: 48 25: 48 26: 48

27: 48 28: 48 29: 48 30: 48 31: 48 32: 48

33: 48 34: 48 35: 48 36: 48 37: 54 38: 54

39: 54 40: 54 41: 54 42: 54 43: 54 44: 54

45: 54 46: 54 47: 54

There are 20 hits at base# 48

There are 11 hits at base# 54

HinfI Gantc 80

8: 77 12: 16 13: 16 14: 16 15: 16 15: 56

15: 77 16: 16 16: 56 16: 77 17: 16 17: 56

17: 77 18: 16 18: 56 18: 77 19: 16 19: 56

19: 77 20: 16 20: 56 20: 77 21: 16 21: 56

21: 77 22: 16 22: 56 22: 77 23: 16 23: 56

23: 77 24: 16 24: 56 24: 77 25: 16 25: 56

25: 77 26: 16 26: 56 26: 77 27: 16 27: 26

27: 56 27: 77 28: 16 28: 56 28: 77 29: 16

29: 56 29: 77 30: 56 31: 16 31: 56 31: 77

32: 16 32: 56 32: 77 33: 16 33: 56 33: 77

34: 16 35: 16 35: 56 35: 77 36: 16 36: 26

36: 56 36: 77 37: 16 38: 16 39: 16 40: 16

41: 16 42: 16 44: 16 45: 16 46: 16 47: 16

48: 46 49: 46

There are 34 hits at base# 16

TfiI Gawtc 21

8: 77 15: 77 16: 77 17: 77 18: 77 19: 77

20: 77 21: 77 22: 77 23: 77 24: 77 25: 77

26: 77 27: 77 28: 77 29: 77 31: 77 32: 77

33: 77 35: 77 36: 77

There are 21 hits at base# 77

MlyI GAGTC 38

12: 16 13: 16 14: 16 15: 16 16: 16 17: 16
18: 16 19: 16 20: 16 21: 16 22: 16 23: 16
24: 16 25: 16 26: 16 27: 16 27: 26 28: 16
29: 16 31: 16 32: 16 33: 16 34: 16 35: 16
36: 16 36: 26 37: 16 38: 16 39: 16 40: 16
41: 16 42: 16 44: 16 45: 16 46: 16 47: 16
48: 46 49: 46

There are 34 hits at base# 16

-"- GACTC 21

15: 56 16: 56 17: 56 18: 56 19: 56 20: 56
21: 56 22: 56 23: 56 24: 56 25: 56 26: 56
27: 56 28: 56 29: 56 30: 56 31: 56 32: 56
33: 56 35: 56 36: 56

There are 21 hits at base# 56

PleI gagtc 38

12: 16 13: 16 14: 16 15: 16 16: 16 17: 16
18: 16 19: 16 20: 16 21: 16 22: 16 23: 16
24: 16 25: 16 26: 16 27: 16 27: 26 28: 16
29: 16 31: 16 32: 16 33: 16 34: 16 35: 16
36: 16 36: 26 37: 16 38: 16 39: 16 40: 16
41: 16 42: 16 44: 16 45: 16 46: 16 47: 16
48: 46 49: 46

There are 34 hits at base# 16

-"- gactc 21

15: 56 16: 56 17: 56 18: 56 19: 56 20: 56
21: 56 22: 56 23: 56 24: 56 25: 56 26: 56
27: 56 28: 56 29: 56 30: 56 31: 56 32: 56
33: 56 35: 56 36: 56

There are 21 hits at base# 56

AlwNI CAGNNNctg 26

15: 68 16: 68 17: 68 18: 68 19: 68 20: 68
21: 68 22: 68 23: 68 24: 68 25: 68 26: 68
27: 68 28: 68 29: 68 30: 68 31: 68 32: 68
33: 68 34: 68 35: 68 36: 68 39: 46 40: 46
41: 46 42: 46

There are 22 hits at base# 68

Table 255: Analysis of frequency of matching REdaptors in actual V genes

A: HpyCH4V in HC at bases 35-56

		Number of mismatches.....											Number		
Id	Ntot	0	1	2	3	4	5	6	7	8	9	10	Cut	Id	Probe
1	**510**	**5**	**11**	**274**	**92**	**61**	**25**	**22**	**11**	**1**	**3**	**5**	**443**	**6-1**	**agttctcccTGCAgctgaactc**
2	**192**	**54**	**42**	**32**	**24**	**15**	**2**	**3**	**10**	**3**	**1**	**6**	**167**	**3-11**	**cactgtatcTGCAaatgaacag**
3	58	19	7	17	6	5	1	0	1	0	2	0	54	3-09	ccctgtatcTGCAaatgaacag
4	**267**	**42**	**33**	**9**	**8**	**8**	**82**	**43**	**22**	**8**	**11**	**1**	**100**	**5-51**	**ccgcctaccTGCAgtggagcag**
5	**250**	**111**	**59**	**41**	**24**	**7**	**5**	**1**	**0**	**0**	**2**	**0**	**242**	**3-15**	**cgctgtatcTGCAaatgaacag**
6	7	0	2	0	1	0	0	0	0	0	4	0	3	7-4.1	cggcatatcTGCAgatctgcag
7	7	0	2	2	0	0	2	1	0	0	0	0	4	3-73	cggcgtatcTGCAaatgaacag
8	26	10	4	1	3	1	2	1	3	1	0	0	19	5-a	ctgcctaccTGCAgtggagcag
9	21	8	2	3	1	6	1	0	0	0	0	0	20	3-49	tcgcctatcTGCAaatgaacag
	1338	249	162	379	149	103	120	71	47	13	23	12	1052		
		249	411	790	939		1162		1280		1316				
						1042		1233		1293		1338			

Id	Probe	dotted probe
6-1	agttctcccTGCAgctgaactc	agttctccc**TGCA**gctgaactc
3-11	cactgtatcTGCAaatgaacag	cac.g.at.....aa.....ag
3-09	ccctgtatcTGCAaatgaacag	ccc.g.at.....aa.....ag
5-51	ccgcctaccTGCAgtggagcag	ccgc..a.......tg..g.ag
3-15	cgctgtatcTGCAaatgaacag	c.c.g.at.....aa.....ag
7-4.1	cggcatatcTGCAgatctgcag	c.gca.at......a.ctg.ag
3-73	cggcgtatcTGCAaatgaacag	c.gcg.at.....aa.....ag
5-a	ctgcctaccTGCAgtggagcag	ctgc..a.......tg..g.ag
3-49	tcgcctatcTGCAaatgaacag	tcgc..at.....aa.....ag

Seqs with the expected RE site only.....1004

(Counts only cases with 4 or fewer mismatches)

Seqs with only an unexpected site..... 0

Seqs with both expected and unexpected.... 48

(Counts only cases with 4 or fewer mismatches)

Seqs with no sites..... 0

B: BlpI in HC

Id	Ntot	0	1	2	3	4	5	6	7	8	Ncut	Name	
1	**133**	**73**	**16**	**11**	**13**	**6**	**9**	**1**	**4**	**0**	**119**	**1-58**	**acatggaGCTGAGCagcctgag**
2	14	11	1	0	0	0	0	1	0	1	12	1-02	acatgga**gctgagc**aggctgag
3	34	17	8	2	6	1	0	0	0	0	0	1-18	acatggagctgaggagcctgag
4	120	50	32	16	10	9	1	1	1	0	2	5-51	acctgcagtggagcagcctgaa
5	55	13	11	10	17	3	1	0	0	0	0	3-15	atctgcaaatgaacagcctgaa
6	340	186	88	41	15	6	3	0	1	0	0	3303	atctgcaaatgaacagcctgag
7	82	25	16	25	12	1	3	0	0	0	0	3-20	atctgcaaatgaacagtctgag
8	3	0	2	0	1	0	0	0	0	0	0	74.1	atctgcagatctgcagcctaaa
9	23	18	2	2	1	0	0	0	0	0	0	3-66	atcttcaaatgaacagcctgag
10	2	1	0	1	0	0	0	0	0	0	0	3-64	atcttcaaatgggcagcctgag
11	**486**	**249**	**78**	**81**	**38**	**21**	**10**	**4**	**4**	**1**	**467**	**4301**	**ccctgaagctgagctctgtgac**
12	16	6	3	1	0	1	1	3	1	0	1	6-1	ccctgcagctgaactctgtgac
13	28	15	8	2	2	1	0	0	0	0	0	2-70	tccttacaatgaccaacatgga
14	2	0	2	0	0	0	0	0	0	0	0	2-26	tccttaccatgaccaacatgga
											601		

Name	Full sequence	Dot mode
1-58	acatggaGCTGAGCagcctgag	acatggaGCTGAGCagcctgag
1-02	acatgga**gctgagc**aggctgag	..............g.....
1-18	acatggagctgaggagcctgag	............g........
5-51	acctgcagtggagcagcctgaa	..c..c..tg..........a
3-15	atctgcaaatgaacagcctgaa	.tc..c.aa...a........a
3-30.3	atctgcaaatgaacagcctgag	.tc..c.aa...a.........
3-20	atctgcaaatgaacagtctgag	.tc..c.aa...a...t.....
7-4.1	atctgcagatctgcagcctaaa	.tc..c..a.ct.......a.a
3-66	atcttcaaatgaacagcctgag	.tc.tc.aa...a.........
3-64	atcttcaaatgggcagcctgag	.tc.tc.aa..g..........
4-30.1	ccctgaagctgagctctgtgac	c.c..a........tctg...c
6-1	ccctgcagctgaactctgtgac	c.c..c......a.tctg...c
2-70	tccttacaatgaccaacatgga	t.c.tacaa...c..a.a..ga
2-26	tccttaccatgaccaacatgga	t.c.tacca...c..a.a..ga

Seqs with the expected RE site only..... 597 (counting sequences with 4 or fewer mismatches)

Seqs with only an unexpected site..... 2

Seqs with both expected and unexpected.... 2

Seqs with no sites..... 686

C: HpyCH4III, Bst4CI, or TaaI in HC

In scoring whether the RE site of interest is present, only ONs that have 4 or fewer mismatches are counted.

Number of sequences..... 1617

Id	Ntot	0	1	2	3	4	5	6	7	8	Ncut	acngt	acngt
1	**244**	**78**	**92**	**43**	**18**	**10**	**1**	**2**	**0**	**0**	**241**	**102#1,1**	**ccgtgtattACTGTgcgagaga**	ccgtgtattactgtgcgagaga
2	**457**	**69**	**150**	**115**	**66**	**34**	**11**	**8**	**3**	**1**	**434**	**103#2,3**	**ctgtgtattactgtgcgagaga**	.t.....
3	**173**	**52**	**45**	**36**	**22**	**14**	**3**	**0**	**0**	**1**	**169**	**108#3**	**ccgtgtattactgtgcgagagg**	.....g
4	16	0	3	2	2	1	6	0	1	1	8	124#5,1	ccgtgtattactgtgcaacaga	.....a.c...
5	4	0	0	1	0	1	1	0	1	0	2	145#6	ccatgtattactgtgcaagata	..a.....a...t.
6	15	1	0	1	0	6	4	1	1	1	8	158#8	ccgtgtattactgtgcggcaga	.....gc...
7	23	4	8	5	2	2	1	1	0	0	21	205#12	ccacatattactgtgcacacag	..aca.....acacag
8	9	1	1	1	0	3	2	1	0	0	6	226#13	ccacatattactgtgcacggat	..aca.....ac.gat
9	7	1	3	1	1	0	0	1	0	0	6	270#14	ccacgtattactgtgcacggat	..ac.....ac.gat
10	23	7	3	5	5	2	1	0	0	0	22	309#16,	ccttgtattactgtgcaaaaga	..t.....a.a...
11	35	5	10	7	6	3	3	0	1	0	31	313#18,	ctgtgtattactgtgcaagaga	.t.....a.....
12	18	2	3	2	2	6	1	0	2	0	15	315#19	ccgtgtattactgtaccacaga	.....a.c.c...
13	3	1	2	0	0	0	0	0	0	0	3	320#20	ccttgtatcactgtgcgagaga	..t.....c.....
14	**117**	**29**	**23**	**28**	**22**	**8**	**4**	**2**	**1**	**0**	**110**	**323#22**	**ccgtatattactgtgcgaaaga**	....a.....a...
15	**75**	**21**	**25**	**13**	**9**	**1**	**4**	**2**	**0**	**0**	**69**	**330#23,**	**ctgtgtattactgtgcgaaaga**	.t.....a...
16	14	2	2	2	3	0	3	1	1	0	9	349#29	ccgtgtattactgtactagaga	.....a.t.....
17	2	0	0	1	0	0	1	0	0	0	1	372#33	ccgtgtattactgtgctagaga	.....t.....
18	1	0	0	1	0	0	0	0	0	0	1	373#34	ccgtgtattactgtactagaca	.....a.t...c.
19	2	0	0	0	0	0	0	0	0	2	0	3d#36	ctgtgtattactgtaagaaaga	.t.....aa..a...
20	34	4	9	9	4	5	3	0	0	0	31	428#38	ccgtgtattactgtgcgagaaa	.....a.
21	17	5	4	2	2	3	1	0	0	0	16	4302#40	ccgtgtattactgtgccagaga	.....c.....
22	**75**	**15**	**17**	**24**	**7**	**10**	**1**	**1**	**0**	**0**	**73**	**439#44**	**ctgtgtattactgtgcgagaca**	.t.....c.
23	**40**	**14**	**15**	**4**	**5**	**1**	**0**	**1**	**0**	**0**	**39**	**551#48**	**ccatgtattactgtgcgagaca**	..a.....c.
24	**213**	**26**	**56**	**60**	**42**	**20**	**7**	**2**	**0**	**0**	**204**	**5a#49**	**ccatgtattactgtgcgagaAA**	..a.....AA
Group		337	471	363	218	130	58	23	11	6				
Cumulative		337	808	1171	1389	1519	1577	1600	1611	1617				

Seqs with the expected RE site only.....1511

Seqs with only an unexpected site..... 0

Tab 255 D

Seqs with both expected and unexpected.... 8
Seqs with no sites..... 0

Analysis repeated using only 8 best REdaptors

Id	Ntot	0	1	2	3	4	5	6	7	8+		
1	301	78	101	54	32	16	9	10	1	0	281	102#1 ccgtgtattactgtgcgagaga
2	493	69	155	125	73	37	14	11	3	6	459	103#2 ctgtgtattactgtgcgagaga
3	189	52	45	38	23	18	5	4	1	3	176	108#3 ccgtgtattactgtgcgagagg
4	127	29	23	28	24	10	6	5	2	0	114	323#22 ccgtatattactgtgcgaaaga
5	78	21	25	14	11	1	4	2	0	0	72	330#23 ctgtgtattactgtgcgaaaga
6	79	15	17	25	8	11	1	2	0	0	76	439#44 ctgtgtattactgtgcgagaca
7	43	14	15	5	5	3	0	1	0	0	42	551#48 ccatgtattactgtgcgagaca
8	307	26	63	72	51	38	24	14	13	6	250	5a#49 ccatgtattactgtgcgaga

1	102#1	ccgtgtattactgtgcgagaga	ccgtgtattactgtgcgagaga
2	103#2	ctgtgtattactgtgcgagaga	.t..................
3	108#3	ccgtgtattactgtgcgagagg	..................g
4	323#22	ccgtatattactgtgcgaaaga	....a.............a...
5	330#23	ctgtgtattactgtgcgaaaga	.t................a...
6	439#44	ctgtgtattactgtgcgagaca	.t.................c.
7	551#48	ccatgtattactgtgcgagaca	..a................c.
8	5a#49	ccatgtattactgtgcgagaAA	..a................AA

Seqs with the expected RE site only.....1463 / 1617
Seqs with only an unexpected site..... 0
Seqs with both expected and unexpected.... 7
Seqs with no sites..... 0

Table 300: Kappa FR1 GLGs

```
! 1   2   3   4   5   6   7   8   9   10  11  12
  GAC ATC CAG ATG ACC CAG TCT CCA TCC TCC CTG TCT
! 13  14  15  16  17  18  19  20  21  22  23
  GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC  !      O12
  GAC ATC CAG ATG ACC CAG TCT CCA TCC TCC CTG TCT
  GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC  !      O2
  GAC ATC CAG ATG ACC CAG TCT CCA TCC TCC CTG TCT
  GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC  !      O18
  GAC ATC CAG ATG ACC CAG TCT CCA TCC TCC CTG TCT
  GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC  !      O8
  GAC ATC CAG ATG ACC CAG TCT CCA TCC TCC CTG TCT
  GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC  !      A20
  GAC ATC CAG ATG ACC CAG TCT CCA TCC TCC CTG TCT
  GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC  !      A30
  AAC ATC CAG ATG ACC CAG TCT CCA TCT GCC ATG TCT
  GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGT  !      L14
  GAC ATC CAG ATG ACC CAG TCT CCA TCC TCA CTG TCT
  GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGT  !      L1
  GAC ATC CAG ATG ACC CAG TCT CCA TCC TCA CTG TCT
  GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGT  !      L15
  GCC ATC CAG TTG ACC CAG TCT CCA TCC TCC CTG TCT
  GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC  !      L4
  GCC ATC CAG TTG ACC CAG TCT CCA TCC TCC CTG TCT
  GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC  !      L18
  GAC ATC CAG ATG ACC CAG TCT CCA TCT TCC GTG TCT
  GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGT  !      L5
  GAC ATC CAG ATG ACC CAG TCT CCA TCT TCT GTG TCT
  GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGT  !      L19
  GAC ATC CAG TTG ACC CAG TCT CCA TCC TTC CTG TCT
  GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC  !      L8
  GCC ATC CGG ATG ACC CAG TCT CCA TTC TCC CTG TCT
  GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC  !      L23
  GCC ATC CGG ATG ACC CAG TCT CCA TCC TCA TTC TCT
  GCA TCT ACA GGA GAC AGA GTC ACC ATC ACT TGT  !      L9
  GTC ATC TGG ATG ACC CAG TCT CCA TCC TTA CTC TCT
```

GCA TCT ACA GGA GAC AGA GTC ACC ATC AGT TGT ! L24
GCC ATC CAG ATG ACC CAG TCT CCA TCC TCC CTG TCT
GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC ! L11
GAC ATC CAG ATG ACC CAG TCT CCT TCC ACC CTG TCT
GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC ! L12
GAT ATT GTG ATG ACC CAG ACT CCA CTC TCC CTG CCC
GTC ACC CCT GGA GAG CCG GCC TCC ATC TCC TGC ! O11
GAT ATT GTG ATG ACC CAG ACT CCA CTC TCC CTG CCC
GTC ACC CCT GGA GAG CCG GCC TCC ATC TCC TGC ! O1
GAT GTT GTG ATG ACT CAG TCT CCA CTC TCC CTG CCC
GTC ACC CTT GGA CAG CCG GCC TCC ATC TCC TGC ! A17
GAT GTT GTG ATG ACT CAG TCT CCA CTC TCC CTG CCC
GTC ACC CTT GGA CAG CCG GCC TCC ATC TCC TGC ! A1
GAT ATT GTG ATG ACC CAG ACT CCA CTC TCT CTG TCC
GTC ACC CCT GGA CAG CCG GCC TCC ATC TCC TGC ! A18
GAT ATT GTG ATG ACC CAG ACT CCA CTC TCT CTG TCC
GTC ACC CCT GGA CAG CCG GCC TCC ATC TCC TGC ! A2
GAT ATT GTG ATG ACT CAG TCT CCA CTC TCC CTG CCC
GTC ACC CCT GGA GAG CCG GCC TCC ATC TCC TGC ! A19
GAT ATT GTG ATG ACT CAG TCT CCA CTC TCC CTG CCC
GTC ACC CCT GGA GAG CCG GCC TCC ATC TCC TGC ! A3
GAT ATT GTG ATG ACC CAG ACT CCA CTC TCC TCA CCT
GTC ACC CTT GGA CAG CCG GCC TCC ATC TCC TGC ! A23
GAA ATT GTG TTG ACG CAG TCT CCA GGC ACC CTG TCT
TTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC ! A27
GAA ATT GTG TTG ACG CAG TCT CCA GCC ACC CTG TCT
TTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC ! A11
GAA ATA GTG ATG ACG CAG TCT CCA GCC ACC CTG TCT
GTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC ! L2
GAA ATA GTG ATG ACG CAG TCT CCA GCC ACC CTG TCT
GTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC ! L16
GAA ATT GTG TTG ACA CAG TCT CCA GCC ACC CTG TCT
TTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC ! L6
GAA ATT GTG TTG ACA CAG TCT CCA GCC ACC CTG TCT
TTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC ! L20
GAA ATT GTA ATG ACA CAG TCT CCA GCC ACC CTG TCT

TTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC ! L25

GAC ATC GTG ATG ACC CAG TCT CCA GAC TCC CTG GCT

GTG TCT CTG GGC GAG AGG GCC ACC ATC AAC TGC ! B3

GAA ACG ACA CTC ACG CAG TCT CCA GCA TTC ATG TCA

GCG ACT CCA GGA GAC AAA GTC AAC ATC TCC TGC ! B2

GAA ATT GTG CTG ACT CAG TCT CCA GAC TTT CAG TCT

GTG ACT CCA AAG GAG AAA GTC ACC ATC ACC TGC ! A26

GAA ATT GTG CTG ACT CAG TCT CCA GAC TTT CAG TCT

GTG ACT CCA AAG GAG AAA GTC ACC ATC ACC TGC ! A10

GAT GTT GTG ATG ACA CAG TCT CCA GCT TTC CTC TCT

GTG ACT CCA GGG GAG AAA GTC ACC ATC ACC TGC ! A14

Table 302 RERS sites found in Human Kappa FR1 GLGs

		MslI	FokI --> <-- -->		PflFI		BsrI		BsmAI		MnlI	HpyCH4V
VKI												
O12	1-69	3	3	23	12	49		15	18	47	26	36
O2	101-169	103	103	123	112	149		115	118	147	126	136
O18	201-269	203	203	223	212	249		215	218	247	226	236
O8	301-369	303	303	323	312	349		315	318	347	326	336
A20	401-469	403	403	423	412	449		415	418	447	426	436
A30	501-569	503	503	523	512	549		515	518	547	526	536
L14	601-669	603	603		612	649		615	618	647	-	636
L1	701-769	703	703	723	712	749		715	718	747	726	736
L15	801-869	803	803	823	812	849		815	818	847	826	836
L4	901-969	-	903	923	912	949	906	915	918	947	926	936
L18	1001-1069	-	1003		1012	1049	1006	1015	1018	1047	1026	1036
L5	1101-1169	1103	-		1112	1149		1115	1118	1147	-	1136
L19	1201-1269	1203	1203		1212	1249		1215	1218	1247	-	1236
L8	1301-1369	-	1303	1323	1312	1349	1306	1315	1318	1347	-	1336
L23	1401-1469	1403	1403	1408	1412	1449		1415	1418	1447	-	1436
L9	1501-1569	1503	1503	1508 1523	1512	1549		1515	1518	1547	1526	1536
L24	1601-1669	1603		1608 1623	1612	1649		1615	1618	1647	-	1636
L11	1701-1769	1703	1703	1723	1712	1749		1715	1718	1747	1726	1736
L12	1801-1869	1803	1803		1812	1849		1815	1818	1847	-	1836

	MslI	FokI --> <-- -->	PflFI	BsrI	BsmAI	MnlI	HpyCH 4V
VKII							
O11 1901-1969	-	-	-	-	-	1956	-
O1 2001-2069	-	-	-	-		2056	-
A17 2101-2169	-	-	2112	-	2118	2156	-
A1 2201-2269	-	-	2212	-	2218	2256	-
A18 2301-2369	-	-	-	-	-	2356	-
A2 2401-2469	-	-	-	-	-	2456	-
A19 2501-2569	-	-	2512	-	2518	2556	-
A3 2601-2669	-	-	2612	-	2618	2656	-
A23 2701-2769	-	-	-	-	-	2729 2756	-
VKIII							
A27 2801-2869	-	-	2812	-	2818 2839	2860	-
A11 2901-2969	-	-	2912	-	2918 2939	2960	-
L2 3001-3069	-	-	3012	-	3018 3039	3060	-
L16 3101-3169	-	-	3112	-	3118 3139	3160	-
L6 3201-3269	-	-	3212	-	3218 3239	3260	-

	MslI	FokI --> <-- -->	PflFI	BsrI	BsmAI	MnlI	HpyCH 4V
L20 3301-3369	-	-	3312	-	3318 3339	3360	-
L25 3401-3469	-	-	3412	-	3418 3439	3460	-
VKIV							
B3 3501-3569	3503	-	3512	3515	3518 3539	3551<	-
VKV							
B2 3601-3669	-	-	3649	-	3618 3647		-
VKVI							
A26 3701-3769	-	-	3712	-	3718		-
A10 3801-3869	-	-	3812	-	3818		-
A14 3901-3969	-	-	3912	-	3918	3930>	-

Table 302 RERS sites found in Human Kappa FR1 GLGs, continued

	SfaNI	SfcI	HinfI	MlyI --> --> <--	MaeIII Tsp45I same sites	HphI xx38 xx56 xx62	HpaII MspI xx06 xx52
VKI							
O12 1-69	37	41	53	53	55	56	-
O2 101-169	137	141	153	153	155	156	-
O18 201-269	237	241	253	253	255	256	-

		SfaNI	SfcI	HinfI	MlyI --> --> <--	MaeIII Tsp45I same sites	HphI xx38 xx56 xx62	HpaII MspI xx06 xx52
O8	301-369	337	341	353	353	355	356	-
A20	401-469	437	441	453	453	455	456	-
A30	501-569	537	541	553	553	555	556	-
L14	601-669	637	641	653	653	655	656	-
L1	701-769	737	741	753	753	755	756	-
L15	801-869	837	841	853	853	855	856	-
L4	901-969	937	941	953	953	955	956	-
L18	1001-1069	1037	1041	1053	1053	1055	1056	-
L5	1101-1169	1137	1141	1153	1153	1155	1156	-
L19	1201-1269	1237	1241	1253	1253	1255	1256	-
L8	1301-1369	1337	1341	1353	1353	1355	1356	-
L23	1401-1469	1437	1441	1453	1453	1455	1456	1406
L9	1501-1569	1537	1541	1553	1553	1555	1556	1506
L24	1601-1669	1637	1641	1653	1653	1655	1656	
L11	1701-1769	1737	1741	1753	1753	1755	1756	
L12	1801-1869	1837	1841	1853	1853	1855	1856	
VKII								
O11	1901-1969	-	-	1918	1918	1937	1938	1952
O1	2001-2069	-	-	2018	2018	2037	2038	2052
A17	2101-2169	-	-	2112	2112	2137	2138	2152
A1	2201-2269	-	-	2212	2212	2237	2238	2252

	SfaNI	SfcI	HinfI	MlyI --> --> <--	MaeIII Tsp45I same sites	HphI xx38 xx56 xx62	HpaII MspI xx06 xx52
A18 2301-2369	-	-	2318	2318	2337	2338	2352
A2 2401-2469	-	-	2418	2418	2437	2438	2452
A19 2501-2569	-	-	2512	2512	2537	2538	2552
A3 2601-2669	-	-	2612	2612	2637	2638	2652
A23 2701-2769	-	-	2718	2718	2737	2731* 2738*	-
VKIII							
A27 2801-2869	-	-	-	-			-
A11 2901-2969	-	-	-	-			-
L2 3001-3069	-	-	-	-			-
L16 3101-3169	-	-	-	-			-
L6 3201-3269	-	-	-	-			-
L20 3301-3369	-	-	-	-			-
L25 3401-3469	-	-	-	-			-
VKIV							
B3 3501-3569	-	-	3525	3525			-
VKV							
B2 3601-3669	-	-	3639	3639			-
VKVI							
A26 3701-3769	-	-	3712 3739	3712 3739	3737 3755	3756 3762	-
A10 3801-3869	-	-	3812 3839	3812 3839	3837 3855	3856 3862	-
A14 3901-3969	-	-	3939	3939	3937 3955	3956 3962	-

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Table 302 RERS sites found in Human Kappa FR1, continued

		BsaJI xx29 xx42 xx43	BssKI (NstNI) xx22 xx30 xx43	BpmI xx20 xx41 xx44 --> --> <--	BsrFI Cac8I NaeI NgoMI V	HaeII I	Tsp509I
VKI							
O12	1-69	-	-	-	-	-	-
O2	101-169	-	-	-	-	-	-
O18	201-269	-	-	-	-	-	-
O8	301-369	-	-	-	-	-	-
A20	401-469	-	-	-	-	-	-
A30	501-569	-	-	-	-	-	-
L14	601-669	-	-	-	-	-	-
L1	701-769	-	-	-	-	-	-
L15	801-869	-	-	-	-	-	-
L4	901-969	-	-	-	-	-	-
L18	1001-1069	-	-	-	-	-	-
L5	1101-1169	-	-	-	-	-	-
L19	1201-1269	-	-	-	-	-	-
L8	1301-1369	-	-	-	-	-	-
L23	1401-1469	-	-	-	-	-	-
L9	1501-1569	-	-	-	-	-	-
L24	1601-1669	-	-	-	-	-	-

	BsaJI xx29 xx42 xx43	BssKI (NstNI) xx22 xx30 xx43	BpmI xx20 xx41 xx44 --> --> <--	BsrFI Cac8I NaeI NgoMI V	HaeII I	Tsp509I
L11 1701-1769	-	-	-	-	-	-
L12 1801-1869	-	-	-	-	-	-
VKII						
O11 1901-1969	1942	1943	1944	1951	1954	-
O1 2001-2069	2042	2043	2044	2051	2054	-
A17 2101-2169	2142	-	-	2151	2154	-
A1 2201-2269	2242	-	-	2251	2254	-
A18 2301-2369	2342	2343	-	2351	2354	-
A2 2401-2469	2442	2443	-	2451	2454	-
A19 2501-2569	2542	2543	2544	2551	2554	-
A3 2601-2669	2642	2643	2644	2651	2654	-
A23 2701-2769	2742	-	-	2751	2754	-
VKIII						
A27 2801-2869	2843	2822 2843	2820 2841	-	-	2803
A11 2901-2969	2943	2943	2920 2941	-	-	2903
L2 3001-3069	3043	3043	3041	-	-	-
L16 3101-3169	3143	3143	3120 3141	-	-	-
L6 3201-3269	3243	3243	3220 3241	-	-	3203
L20 3301-3369	3343	3343	3320 3341	-	-	3303

	BsaJI xx29 xx42 xx43	BssKI (NstNI) xx22 xx30 xx43	BpmI xx20 xx41 xx44 --> --> <--	BsrFI Cac8I NaeI NgoMI V	HaeII I	Tsp509I
L25 3401-3469	3443	3443	3420 3441	-	-	3403
VKIV						
B3 3501-3569	3529	3530	3520	-	3554	
VKV						
B2 3601-3669		3643	3620 3641	-	-	
VKVI						
A26 3701-3769		-	3720	-	-	3703
A10 3801-3869		-	3820	-	-	3803
A14 3901-3969	3943	3943	3920 3941	-	-	-

Table 400 Lambda FR1 GLG sequences

! VL1

CAG TCT GTG CTG ACT CAG CCA CCC TCG GTG TCT GAA
GCC CCC AGG CAG AGG GTC ACC ATC TCC TGT ! 1a
cag tct gtg ctg acG cag ccG ccc tcA gtg tct gGG
gcc ccA Ggg cag agg gtc acc atc tcc tgC ! 1e
cag tct gtg ctg act cag cca ccc tcA gCg tct gGG
Acc ccc Ggg cag agg gtc acc atc tcT tgt ! 1c
cag tct gtg ctg act cag cca ccc tcA gCg tct gGG
Acc ccc Ggg cag agg gtc acc atc tcT tgt ! 1g
cag tct gtg Ttg acG cag ccG ccc tcA gtg tct gCG
gcc ccA GgA cag aAg gtc acc atc tcc tgC ! 1b

! VL2

CAG TCT GCC CTG ACT CAG CCT CCC TCC GCG TCC GGG
TCT CCT GGA CAG TCA GTC ACC ATC TCC TGC ! 2c
cag tct gcc ctg act cag cct cGc tcA gTg tcc ggg
tct cct gga cag tca gtc acc atc tcc tgc! 2e
cag tct gcc ctg act cag cct Gcc tcc gTg tcT ggg
tct cct gga cag tcG Atc acc atc tcc tgc ! 2a2
cag tct gcc ctg act cag cct ccc tcc gTg tcc ggg
tct cct gga cag tca gtc acc atc tcc tgc ! 2d
cag tct gcc ctg act cag cct Gcc tcc gTg tcT ggg
tct cct gga cag tcG Atc acc atc tcc tgc ! 2b2

! VL3

TCC TAT GAG CTG ACT CAG CCA CCC TCA GTG TCC GTG
TCC CCA GGA CAG ACA GCC AGC ATC ACC TGC! 3r
tcc tat gag ctg act cag cca cTc tca gtg tcA gtg
Gcc cTG gga cag acG gcc agG atT acc tgT ! 3j
tcc tat gag ctg acA cag cca ccc tcG gtg tcA gtg
tcc cca gga caA acG gcc agG atc acc tgc! 3p
tcc tat gag ctg acA cag cca ccc tcG gtg tcA gtg
tcc cTa gga cag aTG gcc agG atc acc tgc ! 3a
tcT tCt gag ctg act cag GAC ccT GcT gtg tcT gtg
Gcc TTG gga cag aca gTc agG atc acA tgc ! 3l

tcc tat gTg ctg act cag cca ccc tca gtg tcA gtg
Gcc cca gga Aag acG gcc agG atT acc tgT ! 3h
tcc tat gag ctg acA cag cTa ccc tcG gtg tcA gtg
tcc cca gga cag aca gcc agG atc acc tgc ! 3e
tcc tat gag ctg aTG cag cca ccc tcG gtg tcA gtg
tcc cca gga cag acG gcc agG atc acc tgc ! 3m
tcc tat gag ctg acA cag cca Tcc tca gtg tcA gtg
tcT ccG gga cag aca gcc agG atc acc tgc ! V2-19

! VL4

CTG CCT GTG CTG ACT CAG CCC CCG TCT GCA TCT GCC
TTG CTG GGA GCC TCG ATC AAG CTC ACC TGC ! 4c
cAg cct gtg ctg act caA TcA TcC tct gcC tct gcT
tCC ctg gga Tcc tcg Gtc aag ctc acc tgc ! 4a
cAg cTt gtg ctg act caA TcG ccC tct gcC tct gcc
tCC ctg gga gcc tcg Gtc aag ctc acc tgc ! 4b

! VL5

CAG CCT GTG CTG ACT CAG CCA CCT TCC TCC TCC GCA
TCT CCT GGA GAA TCC GCC AGA CTC ACC TGC ! 5e
cag Gct gtg ctg act cag ccG Gct tcc CTc tcT gca
tct cct gga gCa tcA gcc agT ctc acc tgc ! 5c
cag cct gtg ctg act cag cca Tct tcc CAT tcT gca
tct Tct gga gCa tcA gTc aga ctc acc tgc ! 5b

! VL6

AAT TTT ATG CTG ACT CAG CCC CAC TCT GTG TCG GAG
TCT CCG GGG AAG ACG GTA ACC ATC TCC TGC ! 6a

! VL7

CAG ACT GTG GTG ACT CAG GAG CCC TCA CTG ACT GTG
TCC CCA GGA GGG ACA GTC ACT CTC ACC TGT ! 7a
cag Gct gtg gtg act cag gag ccc tca ctg act gtg
tcc cca gga ggg aca gtc act ctc acc tgt ! 7b

! VL8

CAG ACT GTG GTG ACC CAG GAG CCA TCG TTC TCA GTG
TCC CCT GGA GGG ACA GTC ACA CTC ACT TGT ! 8a

! VL9

CAG CCT GTG CTG ACT CAG CCA CCT TCT GCA TCA GCC
TCC CTG GGA GCC TCG GTC ACA CTC ACC TGC ! 9a

! VL10

CAG GCA GGG CTG ACT CAG CCA CCC TCG GTG TCC AAG
GGC TTG AGA CAG ACC GCC ACA CTC ACC TGC ! 10a

Table 405 RERSs found in human lambda FR1 GLGs

```
! There are 31 lambda GLGs
MlyI NnnnnnGACTC                    25
  1:  6    3:  6    4:  6    6:  6    7:  6    8:  6
  9:  6   10:  6   11:  6   12:  6   15:  6   16:  6
 20:  6   21:  6   22:  6   23:  6   23: 50   24:  6
 25:  6   25: 50   26:  6   27:  6   28:  6   30:  6
 31:  6
 There are  23 hits at base#  6

-"-  GAGTCNNNNNn                    1
 26: 34

MwoI GCNNNNNnngc                    20
  1:  9    2:  9    3:  9    4:  9   11:  9   11: 56
 12:  9   13:  9   14:  9   16:  9   17:  9   18:  9
 19:  9   20:  9   23:  9   24:  9   25:  9   26:  9
 30:  9   31:  9
 There are  19 hits at base#  9
HinfI Gantc                         27
  1: 12    3: 12    4: 12    6: 12    7: 12    8: 12
  9: 12   10: 12   11: 12   12: 12   15: 12   16: 12
 20: 12   21: 12   22: 12   23: 12   23: 46   23: 56
 24: 12   25: 12   25: 56   26: 12   26: 34   27: 12
 28: 12   30: 12   31: 12
 There are  23 hits at base# 12
PleI gactc                          25
  1: 12    3: 12    4: 12    6: 12    7: 12    8: 12
  9: 12   10: 12   11: 12   12: 12   15: 12   16: 12
 20: 12   21: 12   22: 12   23: 12   23: 56   24: 12
 25: 12   25: 56   26: 12   27: 12   28: 12   30: 12
 31: 12
 There are  23 hits at base# 12

-"-  gagtc                          1
 26: 34
```

DdeI Ctnag 32

1: 14 2: 24 3: 14 3: 24 4: 14 4: 24
5: 24 6: 14 7: 14 7: 24 8: 14 9: 14
10: 14 11: 14 11: 24 12: 14 12: 24 15: 5
15: 14 16: 14 16: 24 19: 24 20: 14 23: 14
24: 14 25: 14 26: 14 27: 14 28: 14 29: 30
30: 14 31: 14

There are 21 hits at base# 14

BsaJI Ccnngg 38

1: 23 1: 40 2: 39 2: 40 3: 39 3: 40
4: 39 4: 40 5: 39 11: 39 12: 38 12: 39
13: 23 13: 39 14: 23 14: 39 15: 38 16: 39
17: 23 17: 39 18: 23 18: 39 21: 38 21: 39
21: 47 22: 38 22: 39 22: 47 26: 40 27: 39
28: 39 29: 14 29: 39 30: 38 30: 39 30: 47
31: 23 31: 32

There are 17 hits at base# 39

There are 5 hits at base# 38

There are 5 hits at base# 40 Makes cleavage ragged.

MnlI cctc 35

1: 23 2: 23 3: 23 4: 23 5: 23 6: 19
6: 23 7: 19 8: 23 9: 19 9: 23 10: 23
11: 23 13: 23 14: 23 16: 23 17: 23 18: 23
19: 23 20: 47 21: 23 21: 29 21: 47 22: 23
22: 29 22: 35 22: 47 23: 26 23: 29 24: 27
27: 23 28: 23 30: 35 30: 47 31: 23

There are 21 hits at base# 23

There are 3 hits at base# 19

There are 3 hits at base# 29

There are 1 hits at base# 26

There are 1 hits at base# 27 These could make cleavage ragged.

-"- gagg 7

1: 48 2: 48 3: 48 4: 48 27: 44 28: 44

29: 44

BssKI Nccngg 39

1: 40 2: 39 3: 39 3: 40 4: 39 4: 40
5: 39 6: 31 6: 39 7: 31 7: 39 8: 39
9: 31 9: 39 10: 39 11: 39 12: 38 12: 52
13: 39 13: 52 14: 52 16: 39 16: 52 17: 39
17: 52 18: 39 18: 52 19: 39 19: 52 21: 38
22: 38 23: 39 24: 39 26: 39 27: 39 28: 39
29: 14 29: 39 30: 38

There are 21 hits at base# 39

There are 4 hits at base# 38

There are 3 hits at base# 31

There are 3 hits at base# 40 Ragged

BstNI CCwgg 30

1: 41 2: 40 5: 40 6: 40 7: 40 8: 40
9: 40 10: 40 11: 40 12: 39 12: 53 13: 40
13: 53 14: 53 16: 40 16: 53 17: 40 17: 53
18: 40 18: 53 19: 53 21: 39 22: 39 23: 40
24: 40 27: 40 28: 40 29: 15 29: 40 30: 39

There are 17 hits at base# 40

There are 7 hits at base# 53

There are 4 hits at base# 39

There are 1 hits at base# 41 Ragged

PspGI ccwgg 30

1: 41 2: 40 5: 40 6: 40 7: 40 8: 40
9: 40 10: 40 11: 40 12: 39 12: 53 13: 40
13: 53 14: 53 16: 40 16: 53 17: 40 17: 53
18: 40 18: 53 19: 53 21: 39 22: 39 23: 40
24: 40 27: 40 28: 40 29: 15 29: 40 30: 39

There are 17 hits at base# 40

There are 7 hits at base# 53

There are 4 hits at base# 39

There are 1 hits at base# 41

ScrFI CCngg 39

1: 41 2: 40 3: 40 3: 41 4: 40 4: 41
5: 40 6: 32 6: 40 7: 32 7: 40 8: 40
9: 32 9: 40 10: 40 11: 40 12: 39 12: 53
13: 40 13: 53 14: 53 16: 40 16: 53 17: 40
17: 53 18: 40 18: 53 19: 40 19: 53 21: 39
22: 39 23: 40 24: 40 26: 40 27: 40 28: 40
29: 15 29: 40 30: 39

There are 21 hits at base# 40

There are 4 hits at base# 39

There are 3 hits at base# 41

MaeIII gtnac 16

1: 52 2: 52 3: 52 4: 52 5: 52 6: 52
7: 52 9: 52 26: 52 27: 10 27: 52 28: 10
28: 52 29: 10 29: 52 30: 52

There are 13 hits at base# 52

Tsp45I gtsac 15

1: 52 2: 52 3: 52 4: 52 5: 52 6: 52
7: 52 9: 52 27: 10 27: 52 28: 10 28: 52
29: 10 29: 52 30: 52

There are 12 hits at base# 52

HphI tcacc 26

1: 53 2: 53 3: 53 4: 53 5: 53 6: 53
7: 53 8: 53 9: 53 10: 53 11: 59 13: 59
14: 59 17: 59 18: 59 19: 59 20: 59 21: 59
22: 59 23: 59 24: 59 25: 59 27: 59 28: 59
30: 59 31: 59

There are 16 hits at base# 59

There are 10 hits at base# 53

BspMI ACCTGCNNNNn 14

11: 61 13: 61 14: 61 17: 61 18: 61 19: 61

20: 61 21: 61 22: 61 23: 61 24: 61 25: 61

30: 61 31: 61

There are 14 hits at base# 61 Goes into CDR1

Table 500: h3401-h2 captured Via CJ with BsmAI

```
!  1   2   3   4   5   6   7   8   9  10  11  12  13  14  15
!  S   A   Q   D   I   Q   M   T   Q   S   P   A   T   L   S
  aGT GCA Caa gac atc cag atg acc cag tct cca gcc acc ctg tct
5 !  ApaLI...                                  a gcc acc !
L25,L6,L20,L2,L16,A11
!  Extender.....................................Bridge...

! 16  17  18  19  20  21  22  23  24  25  26  27  28  29  30
10 !  V   S   P   G   E   R   A   T   L   S   C   R   A   S   Q
  gtg tct cca ggg gaa agg gcc acc ctc tcc tgc agg gcc agt cag

! 31  32  33  34  35  36  37  38  39  40  41  42  43  44  45
!  S   V   S   N   N   L   A   W   Y   Q   Q   K   P   G   Q
15   agt gtt agt aac aac tta gcc tgg tac cag cag aaa cct ggc cag

! 46  47  48  49  50  51  52  53  54  55  56  57  58  59  60
!  V   P   R   L   L   I   Y   G   A   S   T   R   A   T   D
  gtt ccc agg ctc ctc atc tat ggt gca tcc acc agg gcc act gat
20

! 61  62  63  64  65  66  67  68  69  70  71  72  73  74  75
!  I   P   A   R   F   S   G   S   G   S   G   T   D   F   T
  atc cca gcc agg ttc agt ggc agt ggg tct ggg aca gac ttc act

25 ! 76  77  78  79  80  81  82  83  84  85  86  87  88  89  90
!  L   T   I   S   R   L   E   P   E   D   F   A   V   Y   Y
  ctc acc atc agc aga ctg gag cct gaa gat ttt gca gtg tat tac

! 91  92  93  94  95  96  97  98  99 100 101 102 103 104 105
30 !  C   Q   R   Y   G   S   S   P   G   W   T   F   G   Q   G
  tgt cag cgg tat ggt agc tca ccg ggg tgg acg ttc ggc caa ggg

! 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
!  T   K   V   E   I   K   R   T   V   A   A   P   S   V   F
35   acc aag gtg gaa atc aaa cga act gtg gct gca cca tct gtc ttc

! 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
!  I   F   P   P   S   D   E   Q   L   K   S   G   T   A   S
  atc ttc ccg cca tct gat gag cag ttg aaa tct gga act gcc tct
40

! 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150
!  V   V   C   L   L   N   N   F   Y   P   R   E   A   K   V
  gtt gtg tgc ctg ctg aat aac ttc tat ccc aga gag gcc aaa gta
```

```
! 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165
!  Q   W   K   V   D   N   A   L   Q   S   G   N   S   Q   E
  cag tgg aag gtg gat aac gcc ctc caa tcg ggt aac tcc cag gag

! 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180
!  S   V   T   E   Q   D   S   K   D   S   T   Y   S   L   S
  agt gtc aca gag cag gac agc aag gac agc acc tac agc ctc agc

! 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195
!  S   T   L   T   L   S   K   A   D   Y   E   K   H   K   V
  agc acc ctg acg ctg agc aaa gca gac tac gag aaa cac aaa gtc

! 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210
!  Y   A   C   E   V   T   H   Q   G   L   S   S   P   V   T
  tac gcc tgc gaa gtc acc cat cag ggc ctg agc tcg cct gtc aca

! 211 212 213 214 215 216 217 218 219 220 221 222 223
!  K   S   F   N   K   G   E   C   K   G   E   F   A
  aag agc ttc aac aaa gga gag tgt aag ggc gaa ttc gc.....
```

Table 501: h3401-d8 KAPPA captured with CJ and *Bsm*AI

```
!  1   2   3   4   5   6   7   8   9  10  11  12  13  14  15
!  S   A   Q   D   I   Q   M   T   Q   S   P   A   T   L   S
  aGT GCA Caa gac atc cag atg acc cag tct cct gcc acc ctg tct
!  ApaLI...Extender.....................a gcc acc !
L25,L6,L20,L2,L16,A11
!                                          A GCC ACC CTG TCT ! L2

!  16  17  18  19  20  21  22  23  24  25  26  27  28  29  30
!  V   S   P   G   E   R   A   T   L   S   C   R   A   S   Q
  gtg tct cca ggt gaa aga gcc acc ctc tcc tgc agg gcc agt cag
! GTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC  !     L2

!  31  32  33  34  35  36  37  38  39  40  41  42  43  44  45
!  N   L   L   S   N   L   A   W   Y   Q   Q   K   P   G   Q
  aat ctt ctc agc aac tta gcc tgg tac cag cag aaa cct ggc cag

!  46  47  48  49  50  51  52  53  54  55  56  57  58  59  60
!  A   P   R   L   L   I   Y   G   A   S   T   G   A   I   G
  gct ccc agg ctc ctc atc tat ggt gct tcc acc ggg gcc att ggt

!  61  62  63  64  65  66  67  68  69  70  71  72  73  74  75
!  I   P   A   R   F   S   G   S   G   S   G   T   E   F   T
  atc cca gcc agg ttc agt ggc agt ggg tct ggg aca gag ttc act
```

```
!  76  77  78  79  80  81  82  83  84  85  86  87  88  89  90
!  L   T   I   S   S   L   Q   S   E   D   F   A   V   Y   F
   ctc acc atc agc agc ctg cag tct gaa gat ttt gca gtg tat ttc

!  91  92  93  94  95  96  97  98  99 100 101 102 103 104 105
!  C   Q   Q   Y   G   T   S   P   P   T   F   G   G   G   T
   tgt cag cag tat ggt acc tca ccg ccc act ttc ggc gga ggg acc

! 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
!  K   V   E   I   K   R   T   V   A   A   P   S   V   F   I
   aag gtg gag atc aaa cga act gtg gct gca cca tct gtc ttc atc

! 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
!  F   P   P   S   D   E   Q   L   K   S   G   T   A   S   V
   ttc ccg cca tct gat gag cag ttg aaa tct gga act gcc tct gtt

! 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150
!  V   C   P   L   N   N   F   Y   P   R   E   A   K   V   Q
   gtg tgc ccg ctg aat aac ttc tat ccc aga gag gcc aaa gta cag

! 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165
!  W   K   V   D   N   A   L   Q   S   G   N   S   Q   E   S
   tgg aag gtg gat aac gcc ctc caa tcg ggt aac tcc cag gag agt

! 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180
!  V   T   E   Q   D   N   K   D   S   T   Y   S   L   S   S
   gtc aca gag cag gac aac aag gac agc acc tac agc ctc agc agc

! 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195
!  T   L   T   L   S   K   V   D   Y   E   K   H   E   V   Y
   acc ctg acg ctg agc aaa gta gac tac gag aaa cac gaa gtc tac

! 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210
!  A   C   E   V   T   H   Q   G   L   S   S   P   V   T   K
   gcc tgc gaa gtc acc cat cag ggc ctt agc tcg ccc gtc acg aag

! 211 212 213 214 215 216 217 218 219 220 221 222 223
!  S   F   N   R   G   E   C   K   K   E   F   V
   agc ttc aac agg gga gag tgt aag aaa gaa ttc gtt t
```

Table 508 Human heavy chains bases 88.1 to 94.2

Number of sequences..... 840

		Number of Mismatchers.....									Probe	
Id	Ntot	0	1	2	3	4	5	6	7	Name	Sequence.....	Dot form.....
1	364	152	97	76	26	7	4	2	0	VHS881-1.1	gctgtgtattactgtgcgag	gctgtgtattactgtgcgag
2	265	150	60	33	13	5	4	0	0	VHS881-1.2	gccgtgtattactgtgcgag	..c..................
3	96	14	34	16	10	5	7	9	1	VHS881-2.1	gccgtatattactgtgcgag	..c..a..............
4	20	0	3	4	9	2	2	0	0	VHS881-4.1	gccgtgtattactgtacgag	..c............a....
5	95	25	36	18	11	2	2	0	1	VHS881-9.1	gccatgtattactgtgcgag	..ca................
	840	341	230	147	69	21	19	11	2			
		341	571	718	787	808	827	838	840			

```
                 88 89 90 91 92 93 94 95 Codon number as in Table 195
                 Recognition.....  Stem..... Loop. Stem.....
(VHS881-1.1) 5'-gctgtgtat|tact-gtgcgag  cAcATccgTg TTgTT cAcggATgTg-3'
(VHS881-1.2) 5'-gccgtgtat|tact-gtgcgag  cAcATccgTg TTgTT cAcggATgTg-3'
(VHS881-2.1) 5'-gccgtatat|tact-gtgcgag  cAcATccgTg TTgTT cAcggATgTg-3'
(VHS881-4.1) 5'-gccgtgtat|tact-gtacgag  cAcATccgTg TTgTT cAcggATgTg-3'
(VHS881-9.1) 5'-gccatgtat|tact-gtgcgag  cAcATccgTg TTgTT cAcggATgTg-3'
                              | site of substrate cleavage

(FOKIact)    5'-cAcATccgTg TTgTT cAcggATgTg-3'

(VHEx881) 5'-AATAgTAgAc TgcAgTgTcc TcAgcccTTA AgcTgTTcAT cTgcAAgTAg-
            AgAgTATTcT TAgAgTTgTc TcTAgAcTTA gTgAAgcg-3'
! note that VHEx881 is the reverse complement of the ON below
!         [RC] 5'-cgCttcacTaag-
!                Scab........
!                Synthetic 3-23 as in Table 206
!                |TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-
!                 XbaI...
!                |aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|t-3'
!                      AflII...
(VHBA881)      5'-cgCttcacTaag-
                 |TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-
                 |aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|tgt gcg ag-3'
(VHBB881)      5'-cgCttcacTaag-
```

```
                    |TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-
                    |aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|tgt Acg ag-3'
(VH881PCR) 5'-cgCttcacTaag|TCT|AGA|gac|aac -3'
```

Table 512: Kappa, bases 12-30

ID	Ntot	0	1	2	3	4	5	6	Name	Sequence.....	Dot Form.....
1	84	40	21	20	1	2	0	0	SK12O12	gacccagtctccatcctcc	gacccagtctccatcctcc
2	32	19	3	6	2	1	0	1	SK12A17	gactcagtctccactctcc	...t.....ct....
3	26	17	8	1	0	0	0	0	SK12A27	gacgcagtctccaggcacc	...g.....gg.a..
4	40	21	18	1	0	0	0	0	SK12A11	gacgcagtctccagccacc	...g.....g..a..
	182	97	50	28	3	3	0	1			
		97	147	175	178	181	181	182			

URE adapters:

```
                        Stem..... Loop. Stem..... Recognition.....
(SzKB1230-O12)       5'-cAcATccgTg TTgTT cAcggATgTg ggAggATggAgAcTgggTc-3'
                [RC] 5'-gacccagtctccatcctcc cAcATccgTg AAcAA cAcggATgTg-3'
                        Recognition..... Stem..... loop. Stem.....
                                           FokI.            FokI.

                        Stem..... Loop. Stem..... Recognition.....
(SzKB1230-A17)       5'-cAcATccgTg TTgTT cAcggATgTg ggAgAgTggAgAcTgAgTc-3'
                [RC] 5'-gactcagtctccactctcc cAcATccgTg AAcAA cAcggATgTg-3'
                        Recognition..... Stem..... loop. Stem.....
                                           FokI.            FokI.

                        Stem..... Loop. Stem..... Recognition.....
(SzKB1230-A27)       5'-cAcATccgTg TTgTT cAcggATgTg ggTgccTggAgAcTgcgTc-3'
                [RC] 5'-gacgcagtctccaggcacc cAcATccgTg AAcAA cAcggATgTg-3'
                        Recognition..... Stem..... loop. Stem.....
                                           FokI.            FokI.

                        Stem..... Loop. Stem..... Recognition.....
(SzKB1230-A11)       5'-cAcATccgTg TTgTT cAcggATgTg ggTggcTggAgAcTgcgTc-3'
                [RC] 5'-gacgcagtctccagccacc cAcATccgTg AAcAA cAcggATgTg-3'
                        Recognition..... Stem..... loop. Stem.....
                                           FokI.            FokI.
```

What happens in the upper strand:

```
(SzKB1230-O12*)                          5'-gac cca gtc|tcc a-tc ctc c-3'
!                                                      | Site of cleavage in substrate
!
(SzKB1230-A17*)                          5'-gac tca gtc|tcc a-ct ctc c-3'
!
(SzKB1230-A27*)                          5'-gac gca gtc|tcc a-gg cac c-3'
!
(SzKB1230-A11*)                          5'-gac gca gtc|tcc a-gc cac c-3'

(kapextURE)      5'-ccTctactctTgTcAcAgTgcAcAA gAc ATc cAg-3'  !sense strand
                    Scab.............ApaLI.

(kapextUREPCR)   5'-ccTctactctTgTcAcAgTg-3'
                    Scab............

(kaBRO1UR)       5'-ggAggATggA cTggATgTcT TgTgcAcTgT gAcAAgAgTA gAgg-3'
!          [RC]  5'-ccTctactctTgTcAcAgTgcAcAA gAc ATc cAg tcc a-tc ctc c-3' ON above is R.C. of this one
(kaBRO2UR)       5'-ggAgAgTggA cTggATgTcT TgTgcAcTgT gAcAAgAgTA gAgg-3'
!          [RC]  5'-ccTctactctTgTcAcAgTgcAcAA gAc ATc cAg tcc a-ct ctc c-3' ON above is R.C. of this one
(kaBRO3UR)       5'-ggTgccTggA cTggATgTcT TgTgcAcTgT gAcAAgAgTA gAgg-3'
!          [RC]  5'-ccTctactctTgTcAcAgTgcAcAA gAc ATc cAg tcc a-gg cac c-3' ON above is R.C. of this one
(kaBRO4UR)       5'-ggTggcTggA cTggATgTcT TgTgcAcTgT gAcAAgAgTA gAgg-3'
!          [RC]  5'-ccTctactctTgTcAcAgTgcAcAA gAc ATc cAg tcc a-gc cac c-3' ON above is R.C. of this one
                    Scab.............ApaLI.
```

```
                |TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-
                |aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|tgt Acg ag-3'
(VH881PCR) 5'-cgCttcacTaag|TCT|AGA|gac|aac -3'
```

Table 512: Kappa, bases 12-30

ID	Ntot	0	1	2	3	4	5	6	Name	Sequence.....	Dot Form.....
1	84	40	21	20	1	2	0	0	SK12O12	gacccagtctccatcctcc	gacccagtctccatcctcc
2	32	19	3	6	2	1	0	1	SK12A17	gactcagtctccactctcc	...t.....ct....
3	26	17	8	1	0	0	0	0	SK12A27	gacgcagtctccaggcacc	...g.....gg.a..
4	40	21	18	1	0	0	0	0	SK12A11	gacgcagtctccagccacc	...g.....g..a..
	182	97	50	28	3	3	0	1			
		97	147	175	178	181	181	182			

URE adapters:

```
                        Stem..... Loop. Stem..... Recognition........
(SzKB1230-O12)       5'-cAcATccgTg TTgTT cAcggATgTg ggAggATggAgAcTgggTc-3'
                [RC] 5'-gacccagtctccatcctcc cAcATccgTg AAcAA cAcggATgTg-3'
                        Recognition........ Stem..... loop. Stem.....
                                             FokI.            FokI.

                        Stem..... Loop. Stem..... Recognition........
(SzKB1230-A17)       5'-cAcATccgTg TTgTT cAcggATgTg ggAgAgTggAgAcTgAgTc-3'
                [RC] 5'-gactcagtctccactctcc cAcATccgTg AAcAA cAcggATgTg-3'
                        Recognition........ Stem..... loop. Stem.....
                                             FokI.            FokI.

                        Stem..... Loop. Stem..... Recognition........
(SzKB1230-A27)       5'-cAcATccgTg TTgTT cAcggATgTg ggTgccTggAgAcTgcgTc-3'
                [RC] 5'-gacgcagtctccaggcacc cAcATccgTg AAcAA cAcggATgTg-3'
                        Recognition........ Stem..... loop. Stem.....
                                             FokI.            FokI.

                        Stem..... Loop. Stem..... Recognition........
(SzKB1230-A11)       5'-cAcATccgTg TTgTT cAcggATgTg ggTggcTggAgAcTgcgTc-3'
                [RC] 5'-gacgcagtctccagccacc cAcATccgTg AAcAA cAcggATgTg-3'
                        Recognition........ Stem..... loop. Stem.....
                                             FokI.            FokI.
```

What happens in the upper strand:

```
(SzKB1230-O12*)                          5'-gac cca gtc|tcc a-tc ctc c-3'
!                                                     | Site of cleavage in substrate
!
(SzKB1230-A17*)                          5'-gac tca gtc|tcc a-ct ctc c-3'
!
(SzKB1230-A27*)                          5'-gac gca gtc|tcc a-gg cac c-3'
!
(SzKB1230-A11*)                          5'-gac gca gtc|tcc a-gc cac c-3'

(kapextURE)     5'-ccTctactctTgTcAcAgTgcAcAA gAc ATc cAg-3' !sense strand
                   Scab.............ApaLI.

(kapextUREPCR) 5'-ccTctactctTgTcAcAgTg-3'
                   Scab............

(kaBRO1UR)    5'-ggAggATggA cTggATgTcT TgTgcAcTgT gAcAAgAgTA gAgg-3'
!        [RC] 5'-ccTctactctTgTcAcAgTgcAcAA gAc ATc cAg tcc a-tc ctc c-3' ON above is R.C. of this one
(kaBRO2UR)    5'-ggAgAgTggA cTggATgTcT TgTgcAcTgT gAcAAgAgTA gAgg-3'
!        [RC] 5'-ccTctactctTgTcAcAgTgcAcAA gAc ATc cAg tcc a-ct ctc c-3' ON above is R.C. of this one
(kaBRO3UR)    5'-ggTgccTggA cTggATgTcT TgTgcAcTgT gAcAAgAgTA gAgg-3'
!        [RC] 5'-ccTctactctTgTcAcAgTgcAcAA gAc ATc cAg tcc a-gg cac c-3' ON above is R.C. of this one
(kaBRO4UR)    5'-ggTggcTggA cTggATgTcT TgTgcAcTgT gAcAAgAgTA gAgg-3'
!        [RC] 5'-ccTctactctTgTcAcAgTgcAcAA gAc ATc cAg tcc a-gc cac c-3' ON above is R.C. of this one
                  Scab.............ApaLI.
```

Table 515 Lambda URE adapters bases 13.3 to 19.3

! Number of sequences..... 128

Id	Ntot	Number of mismatches..... 0	1	2	3	4	5	6	7	8	Name	Sequence.....	Dot form.....
1	58	45	7	1	0	0	0	2	2	1	VL133-2a2	gtctcctggacagtcgatc	gtctcctggacagtcgatc
2	16	10	1	0	1	0	1	1	0	2	VL133-3l	ggccttgggacagacagtc	.g.cttg......a.ag..
3	17	6	0	0	0	4	1	1	5	0	VL133-2c	gtctcctggacagtcagtc	..............ag..
4	37	3	0	10	4	4	3	7	4	2	VL133-1c	ggccccagggcagagggtc	.g.c..a..g...ag.g..
	128	64	8	11	5	8	5	11	11	5			
		64	72	83	88	96	101	112	123	128			

```
!                    Stem...... loop. Stem...... Recognition........
(VL133-2a2)       5'-cAcATccgTg TTgTT cAcggATgTg gATcgAcTgTccAggAgAc-3'
!              [RC] 5'-gtctcctggacagtcgatc cAcATccgTg AAcAA cAcggATgTg-3'
!                    Recognition........ Stem...... Loop. Stem......
!
!                    Stem...... loop. Stem...... Recognition........
(VL133-3l)        5'-cAcATccgTg TTgTT cAcggATgTg gAcTgTcTgTcccAAggcc-3'
!              [RC] 5'-ggccttgggacagacagtc cAcATccgTg AAcAA cAcggATgTg-3'
!                    Recognition........ Stem...... Loop. Stem......
!
!                    Stem...... loop. Stem...... Recognition........
(VL133-2c)        5'-cAcATccgTg TTgTT cAcggATgTg gAcTgAcTgTccAggAgAc-3'
!              [RC] 5'-gtctcctggacagtcagtc cAcATccgTg AAcAA cAcggATgTg-3'
!                    Recognition........ Stem...... Loop. Stem......
!
!                    Stem...... loop. Stem...... Recognition........
(VL133-1c)        5'-cAcATccgTg TTgTT cAcggATgTg gAcccTcTgcccTggggcc-3'
!              [RC] 5'-ggccccagggcagagggtc cAcATccgTg AAcAA cAcggATgTg-3'
```

What happens in the top strand:

```
!                                | site of cleavage in the upper strand
(VL133-2a2*)     5'-g tct cct g|ga cag tcg atc
!
(VL133-3l*)      5'-g gcc ttg g|ga cag aca gtc
!
(VL133-2c*)      5'-g tct cct g|ga cag tca gtc
!
(VL133-1c*)      5'-g gcc cca g|gg cag agg gtc
!
! The following Extenders and Bridges all encode the AA sequence of 2a2 for
codons 1-15
!                                        1
(ON_LamEx133)  5'-ccTcTgAcTgAgT gcA cAg -
!
!             2   3   4   5   6   7   8   9   10  11  12
            AGt gcT TtA acC caA ccG gcT AGT gtT AGC ggT-
!
!             13  14  15
            tcC ccG g !  2a2
!                                       1
(ON_LamB1-133) [RC] 5'-ccTcTgAcTgAgT gcA cAg -
!
!             2   3   4   5   6   7   8   9   10  11  12
            AGt gcT TtA acC caA ccG gcT AGT gtT AGC ggT-
!
!             13  14  15
            tcC ccG g ga cag tcg at-3' !  2a2  N.B. the actual seq is the
!                                              reverse complement of the
!                                              one shown.
!
(ON_LamB2-133) [RC] 5'-ccTcTgAcTgAgT gcA cAg -
!
!             2   3   4   5   6   7   8   9   10  11  12
            AGt gcT TtA acC caA ccG gcT AGT gtT AGC ggT-
!
!             13  14  15
            tcC ccG g ga cag aca gt-3' ! 3l  N.B. the actual seq is the
!                                              reverse complement of the
!                                              one shown.
!
!
(ON_LamB3-133) [RC] 5'-ccTcTgAcTgAgT gcA cAg -
!
!             2   3   4   5   6   7   8   9   10  11  12
            AGt gcT TtA acC caA ccG gcT AGT gtT AGC ggT-
!
!             13  14  15
            tcC ccG g ga cag tca gt -3'! 2c  N.B. the actual seq is the
!                                              reverse complement of the
!                                              one shown.
!
(ON_LamB4-133) [RC] 5'-ccTcTgAcTgAgT gcA cAg -
!
```

```
!            2   3   4   5   6   7   8   9   10  11  12
            AGt gcT TtA acC caA ccG gcT AGT gtT AGC ggT-
!
!            13  14  15
5           tcC ccG g gg cag agg gt-3' ! 1c  N.B. the actual seq is the
!                                             reverse complement of the
!                                             one shown.
!
(ON_Lam133PCR)  5'-ccTcTgAcTgAgT gcA cAg AGt gc-3'
```

Table 525 ONs used in Capture of kappa light chains using CJ method and *Bsm*AI

All ONs are written 5' to 3'.

REdapters (6)

ON_20SK15O12	gggAggATggAgAcTgggTc
ON_20SK15L12	gggAAgATggAgAcTgggTc
ON_20SK15A17	gggAgAgTggAgAcTgAgTc
ON_20SK15A27	gggTgccTggAgAcTgcgTc
ON_20SK15A11	gggTggcTggAgAcTgcgTc
ON_20SK15B3	gggAgTcTggAgAcTgggTc

Bridges (6)

kapbri1O12	gggAggATggAgAcTgggTcATcTggATgTcTTgTgcAcTgTgAcAgAgg
kapbri1L12	gggAAgATggAgAcTgggTcATcTggATgTcTTgTgcAcTgTgAcAgAgg
kapbri1A17	gggAgAgTggAgAcTgggTcATcTggATgTcTTgTgcAcTgTgAcAgAgg
kapbri1A27	gggTgccTggAgAcTgggTcATcTggATgTcTTgTgcAcTgTgAcAgAgg
kapbri1A11	gggTggcTggAgAcTgggTcATcTggATgTcTTgTgcAcTgTgAcAgAgg
kapbri1B3	gggAgTcTggAgAcTgggTcATcTggATgTcTTgTgcAcTgTgAcAgAgg

Extender (5' biotinylated)

kapext1bio	ccTcTgTcAcAgTgcAcAAgAcATccAgATgAcccAgTcTcc

Primers

kaPCRt1	ccTcTgTcAcA**gTgcAc**AAgAc
kapfor	5'-aca ctc tcc cct gtt gaa gct ctt-3'

Table 530

PCR program for amplification of kappa DNA

95°C 5 minutes
95°C 15 seconds
65°C 30 seconds

72°C 1 minute
72°C 7 minutes
4°C hold

Reagents (100 ul reaction):

Template	50 ng
10x turbo PCR buffer	1x
turbo Pfu	4U
dNTPs	200 μM each
kaPCRt1	300 nM
kapfor	300 nM

Table 610: Stuffer used in VH

```
   1 TCCGGAGCTT CAGATCTGTT TGCCTTTTTG TGGGGTGGTG CAGATCGCGT TACGGAGATC
  61 GACCGACTGC TTGAGCAAAA GCCACGCTTA ACTGCTGATC AGGCATGGGA TGTTATTCGC
 121 CAAACCAGTC GTCAGGATCT TAACCTGAGG CTTTTTTTAC CTACTCTGCA AGCAGCGACA
 181 TCTGGTTTGA CACAGAGCGA TCCGCGTCGT CAGTTGGTAG AAACATTAAC ACGTTGGGAT
 241 GGCATCAATT TGCTTAATGA TGATGGTAAA ACCTGGCAGC AGCCAGGCTC TGCCATCCTG
 301 AACGTTTGGC TGACCAGTAT GTTGAAGCGT ACCGTAGTGG CTGCCGTACC TATGCCATTT
 361 GATAAGTGGT ACAGCGCCAG TGGCTACGAA ACAACCCAGG ACGGCCCAAC TGGTTCGCTG
 421 AATATAAGTG TTGGAGCAAA AATTTTGTAT GAGGCGGTGC AGGGAGACAA ATCACCAATC
 481 CCACAGGCGG TTGATCTGTT TGCTGGGAAA CCACAGCAGG AGGTTGTGTT GGCTGCGCTG
 541 GAAGATACCT GGGAGACTCT TTCCAAACGC TATGGCAATA ATGTGAGTAA CTGGAAAACA
 601 CCTGCAATGG CCTTAACGTT CCGGGCAAAT AATTTCTTTG GTGTACCGCA GGCCGCAGCG
 661 GAAGAAACGC GTCATCAGGC GGAGTATCAA AACCGTGGAA CAGAAAACGA TATGATTGTT
 721 TTCTCACCAA CGACAAGCGA TCGTCCTGTG CTTGCCTGGG ATGTGGTCGC ACCCGGTCAG
 781 AGTGGGTTTA TTGCTCCCGA TGGAACAGTT GATAAGCACT ATGAAGATCA GCTGAAAATG
 841 TACGAAAATT TTGGCCGTAA GTCGCTCTGG TTAACGAAGC AGGATGTGGA GGCGCATAAG
 901 GAGTCGTCTA GA
```

Table 620: DNA sequence of pCES5

```
! pCES5  6680  bases = pCes4 with stuffers in CDR1-2 and CDR3 2000.12.13
!
! Ngene =  6680
! Useful REs (cut MAnoLI fewer than 3 times) 2000.06.05
!
! Non-cutters
!Acc65I Ggtacc          AfeI AGCgct           AvrII Cctagg
!BsaBI GATNNnnatc      BsiWI Cgtacg          BsmFI Nnnnnnnnnnnnnnngtccc
!BsrGI Tgtaca          BstAPI GCANNNNntgc    BstBI TTcgaa
!BstZ17I GTAtac        BtrI CACgtg           Ecl136I GAGctc
!EcoRV GATatc          FseI GGCCGGcc         KpnI GGTACc
!MscI TGGcca           NruI TCGcga           NsiI ATGCAt
!PacI TTAATtaa         PmeI GTTTaaac         PmlI CACgtg
!PpuMI RGgwccy         PshAI GACNNnngtc      SacI GAGCTc
!SacII CCGCgg          SbfI CCTGCAgg         SexAI Accwggt
!SgfI GCGATcgc         SnaBI TACgta          SpeI Actagt
!SphI GCATGc           Sse8387I CCTGCAgg     StuI AGGcct
!SwaI ATTTaaat         XmaI Cccggg
!
! cutters
! Enzymes that cut more than     3 times.
!AlwNI CAGNNNctg                5
!BsgI ctgcac                    4
!BsrFI Rccggy                   5
!EarI CTCTTCNnnn                4
!FauI nNNNNNNGCGGG             10
!
! Enzymes that cut from 1 to     3 times.
!
!Eco0109I RGgnccy               3       7  2636  4208
!BssSI Ctcgtg                   1      12
!-"-   Cacgag                   1    1703
!BspHI Tcatga                   3      43   148  1156
!AatII GACGTc                   1      65
!BciVI GTATCCNNNNNN             2     140  1667
!Eco57I CTGAAG                  1     301
!-"-    cttcag                  2    1349
!AvaI Cycgrg                    3     319  2347  6137
!BsiHKAI GWGCWc                 3     401  2321  4245
!HgiAI GWGCWc                   3     401  2321  4245
!BcgI gcannnnnntcg              1     461
!ScaI AGTact                    1     505
```

!PvuI	CGATcg	3	616	3598	5926
!FspI	TGCgca	2	763	5946	
!BglI	GCCNNNNnggc	3	864	2771	5952
!BpmI	CTGGAG	1	898		
!-"-	ctccag	1	4413		
!BsaI	GGTCTCNnnnn	1	916		
!AhdI	GACNNNnngtc	1	983		
!Eam1105I	GACNNNnngtc	1	983		
!DrdI	GACNNNNnngtc	3	1768	6197	6579
!SapI	gaagagc	1	1998		
!PvuII	CAGctg	3	2054	3689	5896
!PflMI	CCANNNNntgg	3	2233	3943	3991
!HindIII	Aagctt	1	2235		
!ApaLI	Gtgcac	1	2321		
!BspMI	Nnnnnnnnngcaggt	1	2328		
!-"-	ACCTGCNNNNn	2	3460		
!PstI	CTGCAg	1	2335		
!AccI	GTmkac	2	2341	2611	
!HincII	GTYrac	2	2341	3730	
!SalI	Gtcgac	1	2341		
!TliI	Ctcgag	1	2347		
!XhoI	Ctcgag	1	2347		
!BbsI	gtcttc	2	2383	4219	
!BlpI	GCtnagc	1	2580		
!EspI	GCtnagc	1	2580		
!SgrAI	CRccggyg	1	2648		
!AgeI	Accggt	2	2649	4302	
!AscI	GGcgcgcc	1	2689		
!BssHII	Gcgcgc	1	2690		
!SfiI	**GGCCNNNNnggcc**	**1**	**2770**		
!NaeI	GCCggc	2	2776	6349	
!NgoMIV	Gccggc	2	2776	6349	
!BtgI	Ccrygg	3	2781	3553	5712
!DsaI	Ccrygg	3	2781	3553	5712
!NcoI	**Ccatgg**	**1**	**2781**		
!StyI	Ccwwgg	3	2781	4205	4472
!MfeI	Caattg	1	2795		
!BspEI	**Tccgga**	**1**	**2861**		
!BglII	**Agatct**	**1**	**2872**		
!BclI	**Tgatca**	**1**	**2956**		
!Bsu36I	**CCtnagg**	**3**	**3004**	**4143**	**4373**
!XcmI	**CCANNNNNnnnntgg**	**1**	**3215**		
!MluI	**Acgcgt**	**1**	**3527**		

```
!HpaI GTTaac                    1    3730
!XbaI Tctaga                    1    3767
!
!AflII Cttaag                   1    3811
!BsmI NGcattc                   1    3821
!-"- GAATGCN                    1    4695
!RsrII CGgwccg                  1    3827
!NheI Gctagc                    1    4166
!BstEII Ggtnacc                 1    4182
!BsmBI CGTCTCNnnnn              2    4188  6625
!-"-  Nnnnnngagacg              1    6673
!ApaI GGGCCc                    1    4209
!BanII GRGCYc                   3    4209  4492  6319
!Bsp120I Gggccc                 1    4209
!PspOMI Gggccc                  1    4209
!BseRI NNnnnnnnnnctcctc         1    4226
!-"-  GAGGAGNNNNNNNNNN          1    4957
!EcoNI CCTNNnnnagg              1    4278
!PflFI GACNnngtc                1    4308
!Tth111I GACNnngtc              1    4308
!KasI Ggcgcc                    2    4327  5967
!BstXI CCANNNNNntgg             1    4415
!NotI GCggccgc                  1    4507
!EagI Cggccg                    1    4508
!BamHI Ggatcc                   1    5169
!BspDI ATcgat                   1    5476
!NdeI CAtatg                    1    5672
!EcoRI Gaattc                   1    5806
!PsiI TTAtaa                    1    6118
!DraIII CACNNNgtg               1    6243
!BsaAI YACgtr                   1    6246
!-------------------------------------------------------------------------
     1     gacgaaaggg cCTCGTGata cgcctatttt tataggttaa tgtcatgata ataatggttt
!                     BssSI.(1/2)
    61     cttaGACGTC aggtggcact tttcggggaa atgtgcgcgg aacccctatt tgtttatttt
!              AatII.
   121     tctaaataca ttcaaatatG TATCCgctca tgagacaata accctgataa atgcttcaat
!                             BciVI..(1 of 2)
   181     aatattgaaa aaggaagagt
! Base #  201 to 1061 = ApR gene from pUC119 with some RE sites removed
!
!            1   2   3   4   5   6   7   8   9  10  11  12  13  14  15
!          fM   S   I   Q   H   F   R   V   A   L   I   P   F   F   A
```

```
   201     atg agt att caa cat ttc cgt gtc gcc ctt att ccc ttt ttt gcg
!
!           16  17  18  19  20  21  22  23  24  25  26  27  28  29  30
!           A   F   C   L   P   V   F   A   H   P   E   T   L   V   K
   246     gca ttt tgc ctt cct gtt ttt gct cac cca gaa acg ctg gtg aaa
!
!           31  32  33  34  35  36  37  38  39  40  41  42  43  44  45
!           V   K   D   A   E   D   Q   L   G   A   R   V   G   Y   I
   291     gta aaa gat gct gaa gat cag ttg ggt gcc cga gtg ggt tac atc
!
!           46  47  48  49  50  51  52  53  54  55  56  57  58  59  60
!           E   L   D   L   N   S   G   K   I   L   E   S   F   R   P
   336     gaa ctg gat ctc aac agc ggt aag atc ctt gag agt ttt cgc ccc
!
!           61  62  63  64  65  66  67  68  69  70  71  72  73  74  75
!           E   E   R   F   P   M   M   S   T   F   K   V   L   L   C
   381     gaa gaa cgt ttt cca atg atg agc act ttt aaa gtt ctg cta tgt
!
!           76  77  78  79  80  81  82  83  84  85  86  87  88  89  90
!           G   A   V   L   S   R   I   D   A   G   Q   E   Q   L   G
   426     ggc gcg gta tta tcc cgt att gac gcc ggg caa gaG CAa ctc ggT
!                                                      BcgI.............
!
!           91  92  93  94  95  96  97  98  99 100 101 102 103 104 105
!           R   R   I   H   Y   S   Q   N   D   L   V   E   Y   S   P
   471     CGc cgc ata cac tat tct cag aat gac ttg gtt gAG TAC Tca cca
!..BcgI......                                          ScaI....
!
!          106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
!           V   T   E   K   H   L   T   D   G   M   T   V   R   E   L
   516     gtc aca gaa aag cat ctt acg gat ggc atg aca gta aga gaa tta
!
!          121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
!           C   S   A   A   I   T   M   S   D   N   T   A   A   N   L
   561     tgc agt gct gcc ata acc atg agt gat aac act gcg gcc aac tta
!
!          136 137 138 139 140 141 142 143 144 145 146 147 148 149 150
!           L   L   T   T   I   G   G   P   K   E   L   T   A   F   L
   606     ctt ctg aca aCG ATC Gga gga ccg aag gag cta acc gct ttt ttg
!                      PvuI.... (1/2)
!
!          151 152 153 154 155 156 157 158 159 160 161 162 163 164 165
!           H   N   M   G   D   H   V   T   R   L   D   R   W   E   P
```

```
   651     cac aac atg ggg gat cat gta act cgc ctt gat cgt tgg gaa ccg
!
!          166 167 168 169 170 171 172 173 174 175 176 177 178 179 180
!           E   L   N   E   A   I   P   N   D   E   R   D   T   T   M
   696     gag ctg aat gaa gcc ata cca aac gac gag cgt gac acc acg atg
!
!          181 182 183 184 185 186 187 188 189 190 191 192 193 194 195
!           P   V   A   M   A   T   T   L   R   K   L   L   T   G   E
   741     cct gta GCA ATG gca aca acg tTG CGC Aaa cta tta act ggc gaa
!                  BsrDI..(1/2)          FspI.... (1/2)
!
!          196 197 198 199 200 201 202 203 204 205 206 207 208 209 210
!           L   L   T   L   A   S   R   Q   Q   L   I   D   W   M   E
   786     cta ctt act cta gct tcc cgg caa caa tta ata gac tgg atg gag
!
!          211 212 213 214 215 216 217 218 219 220 221 222 223 224 225
!           A   D   K   V   A   G   P   L   L   R   S   A   L   P   A
   831     gcg gat aaa gtt gca gga cca ctt ctg cgc tcg gcc ctt ccg gct
!
!          226 227 228 229 230 231 232 233 234 235 236 237 238 239 240
!           G   W   F   I   A   D   K   S   G   A   G   E   R   G   S
   876     ggc tgg ttt att gct gat aaa tCT GGA Gcc ggt gag cgt gGG TCT
!                                      BpmI....(1/2)           BsaI....
!
!          241 242 243 244 245 246 247 248 249 250 251 252 253 254 255
!           R   G   I   I   A   A   L   G   P   D   G   K   P   S   R
   921     Cgc ggt atC ATT GCa gca ctg ggg cca gat ggt aag ccc tcc cgt
! BsaI......         BsrDI...(2/2)
!
!          256 257 258 259 260 261 262 263 264 265 266 267 268 269 270
!           I   V   V   I   Y   T   T   G   S   Q   A   T   M   D   E
   966     atc gta gtt atc tac acG ACg ggg aGT Cag gca act atg gat gaa
!                             AhdI...........
!
!          271 272 273 274 275 276 277 278 279 280 281 282 283 284 285
!           R   N   R   Q   I   A   E   I   G   A   S   L   I   K   H
  1011     cga aat aga cag atc gct gag ata ggt gcc tca ctg att aag cat
!
!          286 287
!           W   .
  1056     tgg taa
  1062                                                  ctgtcagac caagtttact
  1081     catatatact ttagattgat ttaaaacttc atttttaatt taaaaggatc taggtgaaga
```

```
1141     tccttttga taatctcatg accaaaatcc cttaacgtga gttttcgttc cactgagcgt
1201     cagaccccgt agaaaagatc aaaggatctt cttgagatcc ttttttctg cgcgtaatct
1261     gctgcttgca aacaaaaaaa ccaccgctac cagcggtggt ttgtttgccg gatcaagagc
1321     taccaactct ttttccgaag gtaactggct tcagcagagc gcagatacca aatactgtcc
1381     ttctagtgta gccgtagtta ggccaccact tcaagaactc tgtagcaccg cctacatacc
1441     tcgctctgct aatcctgtta ccagtggctg ctgccagtgg cgataagtcg tgtcttaccg
1501     ggttggactc aagacgatag ttaccggata aggcgcagcg gtcgggctga acgggggttt
1561     cgtgcataca gcccagcttg gagcgaacga cctacaccga actgagatac ctacagcgtg
1621     agcattgaga aagcgccacg cttcccgaag ggagaaaggc ggacagGTAT CCggtaagcg
!                                                          BciVI.. (2 of 2)
1681     gcagggtcgg aacaggagag cgCACGAGgg agcttccagg gggaaacgcc tggtatcttt
!                          BssSI.(2/2)
1741     atagtcctgt cgggtttcgc cacctctgac ttgagcgtcg atttttgtga tgctcgtcag
1801     gggggcggag cctatggaaa aacgccagca acgcggcctt tttacggttc ctggcctttt
1861     gctggccttt tgctcACATG Ttctttcctg cgttatcccc tgattctgtg gataaccgta
!                       PciI...
1921     ttaccgcctt tgagtgagct gataccgctc gccgcagccg aacgaccgag cgcagcgagt
1981     cagtgagcga ggaagcgGAA GAGCgcccaa tacgcaaacc gcctctcccc gcgcgttggc
!                         SapI....
2041     cgattcatta atgCAGCTGg cacgacaggt ttcccgactg gaaagcgggc agtgagcgca
!                        PvuII.(1/3)
2101     acgcaatTAA TGTgagttag ctcactcatt aggcacccca ggcTTTACAc tttatgcttc
!              ..-35..          Plac                      ..-10.
2161     cggctcgtat gttgtgtgga attgtgagcg gataacaatt tcacaCAGGA AACAGCTATG
!                                                        M13Rev_seq_primer
2221     ACcatgatta cgCCAAGCTT TGGagccttt tttttggaga ttttcaac
!                       PflMI.......
!                         Hind3.
! signal::linker::CLight
!
!          1   2   3   4   5   6   7   8   9  10  11  12  13  14  15
!         fM   K   K   L   L   F   A   I   P   L   V   V   P   F   Y
2269     gtg aaa aaa tta tta ttc gca att cct tta gtt gtt cct ttc tat
!
!                          Linker.............................. End of FR4
!          16  17  18  19     20  21  22  23  24  25  26  27  28  29  30
!          S   H   S   A      Q   V   Q   L   Q   V   D   L   E   I   K
2314     tct cac aGT GCA    Cag gtc caa CTG CAG GTC GAC CTC GAG atc aaa
!                 ApaLI......           PstI...         XhoI...
!                                         BspMI...
!                                               SalI...
!                                               AccI...(1/2)
!                                               HincII.(1/2)
```

```
!
! Vlight domains could be cloned in as ApaLI-XhoI fragments.
! VL-CL(kappa) segments can be cloned in as ApaLI-AscI fragments. <--------
!
!           Ckappa-------------------------------------------------
!            31  32  33  34  35  36  37  38  39  40  41  42  43  44  45
!            R   G   T   V   A   A   P   S   V   F   I   F   P   P   S
  2359      cgt gga act gtg gct gca cca tct GTC TTC atc ttc ccg cca tct
!                                          BbsI...(1/2)
!
!            46  47  48  49  50  51  52  53  54  55  56  57  58  59  60
!            D   E   Q   L   K   S   G   T   A   S   V   V   C   L   L
  2404      gat gag cag ttg aaa tct gga act gcc tct gtt gtg tgc ctg ctg
!
!            61  62  63  64  65  66  67  68  69  70  71  72  73  74  75
!            N   N   F   Y   P   R   E   A   K   V   Q   W   K   V   D
  2449      aat aac ttc tat ccc aga gag gcc aaa gta cag tgg aag gtg gat
!
!            76  77  78  79  80  81  82  83  84  85  86  87  88  89  90
!            N   A   L   Q   S   G   N   S   Q   E   S   V   T   E   Q
  2494      aac gcc ctc caa tcg ggt aac tcc cag gag agt gtc aca gag cag
!
!            91  92  93  94  95  96  97  98  99 100 101 102 103 104 105
!            D   S   K   D   S   T   Y   S   L   S   S   T   L   T   L
  2539      gac agc aag gac agc acc tac agc ctc agc agc acc ctg acG CTG
!                                                                 EspI...
!
!           106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
!            S   K   A   D   Y   E   K   H   K   V   Y   A   C   E   V
  2584      AGC aaa gca gac tac gag aaa cac aaa GTC TAC gcc tgc gaa gtc
! ...EspI....                                 AccI...(2/2)
!
!           121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
!            T   H   Q   G   L   S   S   P   V   T   K   S   F   N   R
  2629      acc cat cag ggc ctg agt tcA CCG GTg aca aag agc ttc aac agg
!                                   AgeI....(1/2)
!
!           136 137 138 139 140
!            G   E   C   .   .
  2674      gga gag tgt taa taa   GG CGCGCCaatt
!                               AscI.....
!                                BssHII.
!
```

```
2701     ctatttcaag gagacagtca ta

PelB::3-23(stuffed)::CH1::III fusion gene

          1   2   3   4   5   6   7   8   9  10  11  12  13  14  15
        M   K   Y   L   L   P   T   A   A   A   G   L   L   L   L
2723   atg aaa tac cta ttg cct acg gca gcc gct gga ttg tta tta ctc

!-----------------------------------------

      16  17  18  19  20  21  22
      A   A   Q   P   A   M   A
2768 gcG GCC cag ccG GCC atg gcc
       SfiI.............
               NgoMIV..(1/2)
                     NcoI....

                                FR1(DP47/V3-23)---------------
                                23  24  25  26  27  28  29  30
                                 E   V   Q   L   L   E   S   G
2789                            gaa|gtt|CAA|TTG|tta|gag|tct|ggt|
                                      | MfeI  |

      --------------FR1-----------------------------------------
       31  32  33  34  35  36  37  38  39  40  41  42  43  44  45
        G   G   L   V   Q   P   G   G   S   L   R   L   S   C   A
2813  |ggc|ggt|ctt|gtt|cag|cct|ggt|ggt|tct|tta|cgt|ctt|tct|tgc|gct|

      ----FR1-----
       46  47  48
        A   S   G
2858  |gct|TCC|GGA|
          | BspEI |

        Stuffer for CDR1, FR2, and CDR2--------------------------------->
        There are no stop codons in this stuffer.
2867                                                  gcttcAGATC Tgtttgcctt
                                                           BglII..
2887     tttgtggggt ggtgcagatc gcgttacgga gatcgaccga ctgcttgagc aaaagccacg
2947     cttaactgcT GATCAggcat gggatgttat tcgccaaacc agtcgtcagg atcttaacct
                 BclI...
3007     gaggcttttt ttacctactc tgcaagcagc gacatctggt ttgacacaga gcgatccgcg
3067     tcgtcagttg gtagaaacat taacacgttg ggatggcatc aatttgctta atgatgatgg
```

```
  3127     taaaacctgg cagcagccag gctctgccat cctgaacgtt tggctgacca gtatgttgaa
  3187     gcgtaccgta gtggctgccg tacctatgCC Atttgataag TGGtacagcg ccagtggcta
!                                    XcmI.............
  3247     cgaaacaacc caggacggcc caactggttc gctgaatata agtgttggag caaaaatttt
  3307     gtatgaggcg gtgcagggag acaaatcacc aatcccacag gcggttgatc tgtttgctgg
  3367     gaaaccacag caggaggttg tgttggctgc gctggaagat acctgggaga ctctttccaa
  3427     acgctatggc aataatgtga gtaactggaa aacacctgca atggccttaa cgttccgggc
  3487     aaataatttc tttggtgtac cgcaggccgc agcggaagaa ACGCGTcatc aggcggagta
!                                                    MluI..
  3547     tcaaaaccgt ggaacagaaa acgatatgat tgttttctca ccaacgacaa gcgatcgtcc
  3607     tgtgcttgcc tgggatgtgg tcgcacccgg tcagagtggg tttattgctc ccgatggaac
  3667     agttgataag cactatgaag atcagctgaa aatgtacgaa aattttggcc gtaagtcgct
!                               PvuII.
  3727     ctgGTTAACg aagcaggatg tggaggcgca taaggagtcg
!             HpaI..
!             HincII(2/2)
!
!        --------FR3-----------------------------------------------------
!                  4   5   6   7   8   9   10  11  12  13  14  15  16
!                  93  94  95  96  97  98  99 100 101 102 103 104 105
!                  S   R   D   N   S   K   N   T   L   Y   L   Q   M
  3767           |TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|
!                | XbaI  |
!
!        ---FR3------------------------------------------------------------>|
!          17  18  19  20
!         106 107 108 109
!          N   S   L   s    l   s   i   r   s   g
  3806   |aac|agC|TTA|AG t ctg agc att CGG TCC G
!              |AflII |               RsrII..
!
!                  q   h   s   p   t   .
  3834       gg caa cat tct cca aac tga   ccagacga cacaaacggc
  3872       ttacgctaaa tcccgcgcat gggatggtaa agaggtggcg tctttgctgg cctggactca
  3932       tcagatgaag gccaaaaatt ggcaggagtg gacacagcag gcagcgaaac aagcactgac
  3992       catcaactgg tactatgctg atgtaaacgg caatattggt tatgttcata ctggtgctta
  4052       tccagatcgt caatcaggcc atgatccgcg attacccgtt cctggtacgg gaaaatggga
  4112       ctggaaaggg ctattgcctt ttgaaatgaa ccctaaggtg tataaccccc ag
  4164             aa GCTAGC ctgcggcttc
!                    NheI..
!
  4182       G|GTC|ACC|                                          gtc tca agc
!            | BstEII |
```

```
          136 137 138 139 140 141 142 143 144 145 146 147 148 149 150
           A   S   T   K   G   P   S   V   F   P   L   A   P   S   S
 4198      gcc tcc acc aag ggc cca tcg gtc ttc ccc ctg gca ccc tcc tcc

          151 152 153 154 155 156 157 158 159 160 161 162 163 164 165
           K   S   T   S   G   G   T   A   A   L   G   C   L   V   K
 4243      aag agc acc tct ggg ggc aca gcg gcc ctg ggc tgc ctg gtc aag

          166 167 168 169 170 171 172 173 174 175 176 177 178 179 180
           D   Y   F   P   E   P   V   T   V   S   W   N   S   G   A
 4288      gac tac ttc ccc gaa ccg gtg acg gtg tcg tgg aac tca ggc gcc

          181 182 183 184 185 186 187 188 189 190 191 192 193 194 195
           L   T   S   G   V   H   T   F   P   A   V   L   Q   S   S
 4333      ctg acc agc ggc gtc cac acc ttc ccg gct gtc cta cag tcc tca

          196 197 198 199 200 201 202 203 204 205 206 207 208 209 210
           G   L   Y   S   L   S   S   V   V   T   V   P   S   S   S
 4378      gga ctc tac tcc ctc agc agc gta gtg acc gtg ccc tcc agc agc

          211 212 213 214 215 216 217 218 219 220 221 222 223 224 225
           L   G   T   Q   T   Y   I   C   N   V   N   H   K   P   S
 4423      ttg ggc acc cag acc tac atc tgc aac gtg aat cac aag ccc agc

          226 227 228 229 230 231 232 233 234 235 236 237 238
           N   T   K   V   D   K   K   V   E   P   K   S   C
 4468      aac acc aag gtg gac aaG AAA GTT GAG CCC AAA TCT TGT
                              ON-TQHCforw.....................

                                  Poly His linker
                      139 140 141 142 143 144 145 146 147 148 149 150
                       A   A   A   H   H   H   H   H   H   G   A   A
 4507                  GCG GCC GCa cat cat cat cac cat cac ggg gcc gca
                       NotI......
                        EagI....

          151 152 153 154 155 156 157 158 159 160 161 162 163 164 165
           E   Q   K   L   I   S   E   E   D   L   N   G   A   A   .
 4543      gaa caa aaa ctc atc tca gaa gag gat ctg aat ggg gcc gca tag

          Mature III---------------------------------------------->...
          166 167 168 169 170 171 172 173 174 175 176 177 178 179 180
```

```
!       T   V   E   S   C   L   A   K   P   H   T   E   N   S   F
 4588  act gtt gaa agt tgt tta gca aaa cct cat aca gaa aat tca ttt
!
!      181 182 183 184 185 186 187 188 189 190 191 192 193 194 195
!       T   N   V   W   K   D   D   K   T   L   D   R   Y   A   N
 4633  act aac gtc tgg aaa gac gac aaa act tta gat cgt tac gct aac
!
!      196 197 198 199 200 201 202 203 204 205 206 207 208 209 210
!       Y   E   G   C   L   W   N   A   T   G   V   V   V   C   T
 4678  tat gag ggc tgt ctg tgG AAT GCt aca ggc gtt gtg gtt tgt act
!                           BsmI....
!
!      211 212 213 214 215 216 217 218 219 220 221 222 223 224 225
!       G   D   E   T   Q   C   Y   G   T   W   V   P   I   G   L
 4723  ggt gac gaa act cag tgt tac ggt aca tgg gtt cct att ggg ctt
!
!      226 227 228 229 230 231 232 233 234 235 236 237 238 239 240
!       A   I   P   E   N   E   G   G   G   S   E   G   G   G   S
 4768  gct atc cct gaa aat gag ggt ggt ggc tct gag ggt ggc ggt tct
!
!      241 242 243 244 245 246 247 248 249 250 251 252 253 254 255
!       E   G   G   G   S   E   G   G   G   T   K   P   P   E   Y
 4813  gag ggt ggc ggt tct gag ggt ggc ggt act aaa cct cct gag tac
!
!      256 257 258 259 260 261 262 263 264 265 266 267 268 269 270
!       G   D   T   P   I   P   G   Y   T   Y   I   N   P   L   D
 4858  ggt gat aca cct att ccg ggc tat act tat atc aac cct ctc gac
!
!      271 272 273 274 275 276 277 278 279 280 281 282 283 284 285
!       G   T   Y   P   P   G   T   E   Q   N   P   A   N   P   N
 4903  ggc act tat ccg cct ggt act gag caa aac ccc gct aat cct aat
!
!      286 287 288 289 290 291 292 293 294 295 296 297 298 299 300
!       P   S   L   E   E   S   Q   P   L   N   T   F   M   F   Q
 4948  cct tct ctt GAG GAG tct cag cct ctt aat act ttc atg ttt cag
!                  BseRI..(2/2)
!
!      301 302 303 304 305 306 307 308 309 310 311 312 313 314 315
!       N   N   R   F   R   N   R   Q   G   A   L   T   V   Y   T
 4993  aat aat agg ttc cga aat agg cag ggt gca tta act gtt tat acg
!
!      316 317 318 319 320 321 322 323 324 325 326 327 328 329 330
!       G   T   V   T   Q   G   T   D   P   V   K   T   Y   Y   Q
```

```
  5038   ggc act gtt act caa ggc act gac ccc gtt aaa act tat tac cag
!
!        331 332 333 334 335 336 337 338 339 340 341 342 343 344 345
!         Y   T   P   V   S   S   K   A   M   Y   D   A   Y   W   N
  5083   tac act cct gta tca tca aaa gcc atg tat gac gct tac tgg aac
!
!        346 347 348 349 350 351 352 353 354 355 356 357 358 359 360
!         G   K   F   R   D   C   A   F   H   S   G   F   N   E   D
  5128   ggt aaa ttc aga gac tgc gct ttc cat tct ggc ttt aat gaG GAT
!                                                              BamHI..
!
!        361 362 363 364 365 366 367 368 369 370 371 372 373 374 375
!         P   F   V   C   E   Y   Q   G   Q   S   S   D   L   P   Q
  5173   CCa ttc gtt tgt gaa tat caa ggc caa tcg tct gAC CTG Cct caa
! BamHI...                                          BspMI...(2/2)
!
!        376 377 378 379 380 381 382 383 384 385 386 387 388 389 390
!         P   P   V   N   A   G   G   G   S   G   G   G   S   G   G
  5218   cct cct gtc aat gct ggc ggc ggc tct ggt ggt ggt tct ggt ggc
!
!        391 392 393 394 395 396 397 398 399 400 401 402 403 404 405
!         G   S   E   G   G   G   S   E   G   G   G   S   E   G   G
  5263   ggc tct gag ggt ggc ggc tct gag ggt ggc ggt tct gag ggt ggc
!
!        406 407 408 409 410 411 412 413 414 415 416 417 418 419 420
!         G   S   E   G   G   G   S   G   G   G   S   G   S   G   D
  5308   ggc tct gag ggt ggc ggt tcc ggt ggc ggc tcc ggt tcc ggt gat
!
!        421 422 423 424 425 426 427 428 429 430 431 432 433 434 435
!         F   D   Y   E   K   M   A   N   A   N   K   G   A   M   T
  5353   ttt gat tat gaa aaa atg gca aac gct aat aag ggg gct atg acc
!
!        436 437 438 439 440 441 442 443 444 445 446 447 448 449 450
!         E   N   A   D   E   N   A   L   Q   S   D   A   K   G   K
  5398   gaa aat gcc gat gaa aac gcg cta cag tct gac gct aaa ggc aaa
!
!        451 452 453 454 455 456 457 458 459 460 461 462 463 464 465
!         L   D   S   V   A   T   D   Y   G   A   A   I   D   G   F
  5443   ctt gat tct gtc gct act gat tac ggt gct gct ATC GAT ggt ttc
!                                                    BspDI..
!
!        466 467 468 469 470 471 472 473 474 475 476 477 478 479 480
!         I   G   D   V   S   G   L   A   N   G   N   G   A   T   G
```

```
  5488   att ggt gac gtt tcc ggc ctt gct aat ggt aat ggt gct act ggt
!
!        481 482 483 484 485 486 487 488 489 490 491 492 493 494 495
!         D   F   A   G   S   N   S   Q   M   A   Q   V   G   D   G
  5533   gat ttt gct ggc tct aat tcc caa atg gct caa gtc ggt gac ggt
!
!        496 497 498 499 500 501 502 503 504 505 506 507 508 509 510
!         D   N   S   P   L   M   N   N   F   R   Q   Y   L   P   S
  5578   gat aat tca cct tta atg aat aat ttc cgt caa tat tta cct tct
!
!        511 512 513 514 515 516 517 518 519 520 521 522 523 524 525
!         L   P   Q   S   V   E   C   R   P   Y   V   F   G   A   G
  5623   ttg cct cag tcg gtt gaa tgt cgc cct tat gtc ttt ggc gct ggt
!
!        526 527 528 529 530 531 532 533 534 535 536 537 538 539 540
!         K   P   Y   E   F   S   I   D   C   D   K   I   N   L   F
  5668   aaa cCA TAT Gaa ttt tct att gat tgt gac aaa ata aac tta ttc
!           NdeI....
!
!        541 542 543 544 545 546 547 548 549 550 551 552 553 554 555
!         R   G   V   F   A   F   L   L   Y   V   A   T   F   M   Y
  5713   cgt ggt gtc ttt gcg ttt ctt tta tat gtt gcc acc ttt atg tat
!
!        556 557 558 559 560 561 562 563 564 565 566 567 568 569 570
!         V   F   S   T   F   A   N   I   L   R   N   K   E   S   .
  5758   gta ttt tcg acg ttt gct aac ata ctg cgt aat aag gag tct taa
!
!        571
!         .
  5803   taa GAATTC
!           EcoRI.
  5812     actggccgt cgttttacaa cgtcgtgact gggaaaaccc tggcgttacc caacttaatc
  5871    gccttgcagc acatcccccc ttcgccagct ggcgtaatag cgaagaggcc cgcacCGATC
!                                                                  PvuI..
  5931    Gcccttccca acagtTGCGC Agcctgaatg gcgaatGGCG CCtgatgcgg tattttctcc
! ...PvuI... (3/3)         FspI... (2/2)          KasI...(2/2)
  5991    ttacgcatct gtgcggtatt tcacaccgca tataaattgt aaacgttaat attttgttaa
  6051    aattcgcgtt aaatttttgt taaatcagct catttttaa ccaataggcc gaaatcggca
  6111    aaatcccTTA TAAatcaaaa gaatagcccg agatagggtt gagtgttgtt ccagtttgga
!               PsiI...
  6171    acaagagtcc actattaaag aacgtggact ccaacgtcaa agggcgaaaa accgtctatc
  6231    agggcgatgg ccCACtacGT Gaaccatcac ccaaatcaag ttttttgggg tcgaggtgcc
!                    DraIII....
```

```
  6291 gtaaagcact aaatcggaac cctaaaggga gcccccgatt tagagcttga cggggaaaGC
!                                                                    NgoMIV..
  6351     CGGCgaacgt ggcgagaaag gaagggaaga aagcgaaagg agcgggcgct agggcgctgg
!      ..NgoMIV.(2/2)
  6411     caagtgtagc ggtcacgctg cgcgtaacca ccacacccgc cgcgcttaat gcgccgctac
  6471     agggcgcgta ctatggttgc tttgacgggt gcagtctcag tacaatctgc tctgatgccg
  6531     catagttaag ccagccccga cacccgccaa cacccgctga cgcgccctga cgggcttgtc
  6591     tgctcccggc atccgcttac agacaagctg tgaccgtctc cgggagctgc atgtgtcaga
  6651     ggttttcacc gtcatcaccg aaacgcgcga
```

630: Oligonucleotides used to clone CDR1/2 diversity

equences are 5' to 3'.

N_CD1Bsp, 30 bases

```
 c  T  c  A  c  T  g  g  c  T  T  c  c  g  g  A
 3  4  5  6  7  8  9 10 11 12 13 14 15 16 17 18

 c  A  c  T  T  T  c  T  c  T
21 22 23 24 25 26 27 28 29 30
```

N_Br12, 42 bases

```
 A  A  A  c  c  c  A  c  T  c  c  A  A  A  c  c
 3  4  5  6  7  8  9 10 11 12 13 14 15 16 17 18

 T  A  c  c  A  g  g  A  g  c  T  T  g  g  c  g
21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36

 c  c  c  A
39 40 41 42
```

N_CD2Xba, 51 bases

```
 A  A  g  g  c  A  g  T  g  A  T  c  T  A  g  A
 3  4  5  6  7  8  9 10 11 12 13 14 15 16 17 18

 T  A  g  T  g  A  A  g  c  g  A  c  c  T  T  T
21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36

 c  g  g  A  g  T  c  A  g  c  A  T  A
39 40 41 42 43 44 45 46 47 48 49 50 51
```

N_BotXba, 23 bases

	g	g	A	A	g	g	c	A	g	T	g	A	T	c	T	A	g	A
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18

	g	A	T	A	g
5	19	20	21	22	23

10 End Tables